RAPID COMMUNICATION

Granulocyte Colony-Stimulating Factor Rapidly Activates a Distinct STAT-Like Protein in Normal Myeloid Cells

By David J. Tweardy, Timothy M. Wright, Steven F. Ziegler, Heinz Baumann, Arup Chakraborty, Scott M. White, Kevin F. Dyer, and Kristin A. Rubin

Binding of granulocyte colony-stimulating factor (G-CSF) to normal myeloid cells activates the protein tyrosine kinases Lyn and Syk and results in the immediate early upregulation of G-CSFR receptor (R) mRNA. In our studies of the signaling pathways activated by G-CSF that are coupled to proliferation and differentiation of myeloid cells, we examined whether G-CSF activated a latent transcription factor belonging to the STAT protein family. Electrophoretic mobility shift assays (EMSAs) of nuclear extracts from G-CSF-stimulated human myeloid cells showed the rapid activation of a DNA-binding protein that bound to the high-affinity serum-inducible element (hSIE) and migrated with mobility similar to serum inducible factor (SIF)-A (Stat3 homodimer). The G-CSF–stimulated SIF-A complex (G-SIF-A) did not bind to duplex oligonucleotides used to purify and characterize other Stat proteins (Stat1–6). In addition, antibodies raised against Stat1-6 failed to supershift the G-SIF-A complex or interfere with its formation. Based on its binding to the hSIE and lack of antigenic cross-reactivity with other known STAT proteins that bind to this element, it is likely that G-SIF-A is composed of a distinct member of the STAT protein family. EMSAs of whole-cell extracts prepared from cell lines containing full-length and truncated mutants of the G-CSFR showed that activation of G-SIF-A did not correlate with proliferation; rather, optimal activation requires the distal half of the cytosolic domain of the G-CSF that is essential for differentiation. Activation of G-SIF-A, therefore, may be an early G-CSFR–coupled event that is critical for myeloid maturation.

© 1995 by The American Society of Hematology.

MATERIALS AND METHODS

Cells. Human polymorphonuclear neutrophils (PMN) were obtained from peripheral blood samples as described20; cells were ≥95% neutrophils by cytochemical staining. Bone marrow cells were obtained from aspirates of healthy volunteers or from vertebral bodies of organ donors, as described26 (M. Strong, Northwest Tissue Center, Seattle, WA, personal communication, March 9, 1995). Bone marrow mononuclear cells (BMMC) were isolated on a Ficoll-Hypaque gradient and washed with phosphate-buffered saline (PBS). CD34+ cells were isolated from BMMC using a Ceprate LC34 bio-tin-avidin column (CellPro Incorp, Bothell, WA) as described by the manufacturer. CD34+ cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 10% WEHI-3b–conditioned medium as a source of interleukin-3 (IL-3), as described.8 BA/F-HGR-57 (B-57), BA/F-HGR-96 (B-96), and BA/F-HGR-183 (B-183; full-length receptor) were maintained in RPMI-1640 supplemented with 10% FBS and 50 ng/mL recombinant human G-CSF (rhG-CSF, Neupogen). Before electrophoretic mobility shift assay (EMSA), all transductors and activators of transcription (STAT) family have been shown to be activated by several members of the hematopoietin/cytokine receptor family.19,24 STAT proteins characterized thus far range in size, from 77 to 113 kD. These proteins are latent transcription factors that become activated by tyrosine phosphorylation on ligand binding and translocate from the cytoplasm to the nucleus. In the nucleus, each protein binds to a characteristic enhancer sequence and activates gene transcription.

G-CSF has been shown to activate Stat3 (molecular weight [M], of 92,000) in the human myeloid leukemia cell line, AML-193.25 STAT protein activation in normal myeloid cells has not yet been described. We show in this report that G-CSF activates Stat3 in normal myeloid cells a distinct high-affinity serum-inducible element (hSIE)-binding factor G-SIF-A with electrophoretic mobility and DNA binding properties similar to Stat3. Furthermore, we provide evidence that optimal activation of G-SIF-A maps to the region of the G-CSFR required for differentiation.

From the Divisions of Infectious Diseases and Rheumatology, Department of Medicine, University of Pittsburgh School of Medicine and the University of Pittsburgh Cancer Institute, Pittsburgh, PA; Darwin Molecular Corp, Bothell, WA; and Roswell Park Cancer Institute, Buffalo, NY.

Submitted June 14, 1995; accepted September 21, 1995.

Supported in part by American Cancer Society Grant No. DHP-111 and by National Institutes of Health Grants No. AI07353, CA53533 (T.M.W.), and CA26122 (H.B.).

Address reprint requests to David J. Tweardy, MD, W1052 Biomedical Science Tower, University of Pittsburgh Cancer Institute, 200 Lothrop St, Pittsburgh, PA 15213.

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. section 1734 solely to indicate this fact.

© 1995 by The American Society of Hematology.

0006-4971/95/$8612-0045$3.00/0

BAF-series cells were expanded in RPMI-1640 containing 10% FBS and 10% WEHI-3B-conditioned medium. The murine G-CSF-responsive cell line, 32Dc13, was maintained in murine IL-3-containing medium, as described.1 AML-193, a factor-dependent human acute myeloid leukemia cell line, was grown in granulocyte-macrophage colony-stimulating factor (GM-CSF), as described.29 AML-193 cells were shown to proliferate but not to differentiate in response to G-CSF.29

Reagents. rhG-CSF was obtained from Amgen (Thousand Oaks, CA) and rhGM-CSF was obtained from Immunex (Seattle, WA). rhIL-6 was the generous gift of Dr Steve Clark of the Genetics Institute (Cambridge, MA). Recombinant human interferon-y (IFN-y) was obtained from Genentech (South San Francisco, CA). Complimentary oligonucleotides were synthesized based on the published sequences with the addition of GATC at the 5' termini to allow radiolabeling, after annealing, by Klenow fill-in reaction using [32P]-dATP and [35P]-dATP. Sequences of oligonucleotides used in these studies are listed in Table I. Stat1, Stat2, Stat3, Stat4, and Stat6 rabbit polyclonal antibody were obtained from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). Stat1 polyclonal antibody was raised against the C-terminal end of human Stat1, amino acids 716 to 739. Stat2 antisera was raised against the C-terminal end of human Stat2, amino acids 828 to 847. Stat3 antisera was raised against the C-terminal end of murine Stat3, amino acids 750 to 769. Stat4 antisera was raised against the C-terminal end of murine Stat4, amino acids 730 to 749. Stat6 antisera was raised against the C-terminal end of human Stat6, amino acids 828 to 847. Stat5 antibody30 was the generous gift of Dr Hiroshi Wako (DNAX Research Institute, Palo Alto, CA).

EMSA. Cells (≈106) in suspension were incubated in 1 ml PBS with or without cytokine at 37°C. Adherent cells (H-35 and HepG2) were grown in 10-cm plastic tissue culture dishes, incubated with cytokine in medium or PBS, washed, and harvested by scraping with a rubber policeman. Whole cell, cytoplasmic, and nuclear extracts were prepared and EMSAs were performed on 4% native polyacrylamide gels as described.31,32

Tritiated-histidine incorporation assay. Cells (106/microtiter well) were incubated in triplicate in RPMI-1640 media containing 10% fetal bovine serum with or without G-CSF (Neupogen) or WEHI-3B-conditioned media as a source of murine IL-3. Wells were pulsed with 0.5 μCi of [3H]-thymidine 18 hours before harvesting on day 3 onto glass fiber filters. The filters were counted by liquid scintillation and incorporation was measured as counts per minute.

RESULTS

G-CSF stimulated a DNA-binding protein that migrated with mobility similar to SIF-A in human myeloid cells. EMSA of whole-cell extracts of hepatoma cells H-35 and HepG2 stimulated with IL-6 showed three protein complexes that bound the radiolabeled hSIE duplex oligonucleotide (m67)32 that have previously been designated SIF-A, SIF-B, and SIF-C30 (Fig 1A). Stimulation of freshly isolated human neutrophils with G-CSF activated a DNA-binding protein that shifted hSIE on EMSA, forming a complex that migrated with mobility similar to SIF-A (Fig 1), hereafter designated G-SIF-A. Stimulation of neutrophils and hepatoma cells with IFN-y gave a characteristic SIF-C protein complex.14,30 Kinetic studies showed that G-SIF-A activation in neutrophils peaked within 15 to 30 minutes and decreased thereafter, returning to baseline by 4 hours (Fig 1B). G-SIF-A activation was detected at concentrations of G-CSF as low as 1 ng/mL and peaked at 30 ng/mL, which is well within the physiologic concentration range53 (Fig 1C). In contrast to G-CSF, GM-CSF stimulation for up to 3 hours did not activate G-SIF-A in neutrophils (Fig 1D). Analysis of nuclear and cytosolic extracts from G-CSF-stimulated neutrophils showed nuclear localization of G-SIF-A within 1 minute, with greater activity within the nucleus than cytoplasm at 30 minutes (Fig 1E). Similar to neutrophils, G-CSF, but not GM-CSF, activated G-SIF-A in both normal BMMC and purified CD34+ bone marrow cells (Fig 2A). The kinetics of activation of G-SIF-A in bone marrow cells were similar to those in neutrophils (Fig 2B).

G-SIF-A contains a distinct STAT-like protein. Two STAT proteins, Stat1 and Stat3, are known to bind hSIE.14,17 Recent studies have indicated that the IL-6–stimulated SIF-A protein complex is composed of a Stat3 homodimer; the SIF-C complex is a Stat1 homodimer; and the SIF-B complex is a heterodimer of Stat1 and Stat3.14,30 Four additional STAT proteins have been cloned, including Stat2 (ISGF-3, p113),13 Stat4 (MIF, p92),20 Stat5 (MGF),20,22 and Stat6 (IL4-Stat).20 We examined whether G-SIF-A is capable of binding to representative duplex oligonucleotides used to purify these other STAT proteins (Table I). As shown in Fig 3A, G-SIF-A did not shift the GBP GAS,15 ISRE,15 IRF-1 GAS,15 PRE, an element within the bovine casein promoter,18 or an element within the Fc γ receptor I promoter.20 In addition, among these duplex oligonucleotides, only the hSIE could compete with radiolabeled hSIE for binding to G-SIF-A (Fig 3B). In separate experiments performed under identical EMSA conditions, we showed that each duplex oligonucleotide listed in Table I was bound and shifted by its appropriate STAT protein(s) (data not shown). These results suggested that G-SIF-A most closely resembles Stat3, sharing similar electrophoretic mobility and binding element specificity.

To determine if G-SIF-A was distinct from Stat3, we ex-

<table>
<thead>
<tr>
<th>STAT</th>
<th>Oligonucleotide</th>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (p91/p84)</td>
<td>1</td>
<td>GBP-GAS</td>
<td>GATCTTCAAGTTTCATATCTAATCAGGATC</td>
</tr>
<tr>
<td>2 (p91/p84, p113)</td>
<td>2</td>
<td>ISRE</td>
<td>GATCATAACACAGGAAATA GAACTTAAGAAGACATGATC</td>
</tr>
<tr>
<td>3 (p92)</td>
<td>3</td>
<td>hSIE</td>
<td>GATCCATTTCCGTAATCGATC</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>IRF1-GAS</td>
<td>GATCCTAGAGCCTTAGTTCCCGGAATGAGCTAGGATC</td>
</tr>
<tr>
<td>5 (MGF)</td>
<td>b</td>
<td>PRE</td>
<td>GATCAGATTCTGAATTTCAAATCGATC</td>
</tr>
<tr>
<td>6 (IL-4 Stat)</td>
<td>6</td>
<td>—</td>
<td>GATCGTATTTCCGAAAGGAAAGAGATC</td>
</tr>
</tbody>
</table>

www.bloodjournal.orgFrom August 30, 2017. By guest. For personal use only.
G-CSF ACTIVATION OF A DISTINCT STAT-LIKE PROTEIN

Fig 1. Activation of a SIF-A protein complex in extracts of G-CSF–stimulated neutrophils. In (A), H-35 and HepG2 cells were incubated in IL-6 (1,000 U/ml; 16) or IFN-γ (1,000 U/ml; 1y) for 15 minutes; neutrophils were incubated for 15 minutes in PBS alone (O) or G-CSF (1,000 ng/ml; G) or IFN-γ (1,000 U/ml; 1y). Whole cell extracts were prepared and analyzed by EMSA using radiolabeled hSIE duplex oligonucleotide. In (B), neutrophils were stimulated with G-CSF (1,000 ng/ml) for the times indicated before whole-cell extraction and EMSA using radiolabeled hSIE duplex oligonucleotide. In (C), neutrophils were stimulated with G-CSF (100 ng/ml) for the times indicated before whole-cell extraction and EMSA with radiolabeled hSIE. In (D), neutrophils were stimulated with G-CSF (100 ng/ml) or GM-CSF (100 ng/ml) for the times indicated before whole-cell extraction and EMSA with radiolabeled hSIE. In (E), neutrophils were stimulated with G-CSF (100 ng/ml) for the times indicated before extraction of cytoplasmic proteins followed by nuclear extraction. EMSA was performed on each set of extracts using radiolabeled hSIE.

The addition of one or three micrograms of this antibody completely supershifted the SIF-A band in samples containing extracts from IL-6–stimulated HepG2 cells (Fig 4A). However, the addition of up to 3 µg of this antibody failed to affect either the mobility or the formation of the G-SIF-A band in neutrophils, BMMC, and CD34+ cells (Fig 4A and B). The addition of polyclonal antibodies against Stat1, Stat2, Stat4, Stat5, and Stat6 in an amount sufficient to supershift its respective Stat protein failed to supershift G-SIF-A or affect its formation (data not shown). These results suggested that G-SIF-A in normal human myeloid cells contains a STAT-like protein that is immunologically distinct from Stat3, as well as Stat1, 2, 4, 5, and 6.

In contrast to the results using extracts from normal human myeloid cells, the G-SIF-A band formed by extracts from G-CSF–stimulated AML-193 cells (human acute myeloid leukemic cell line) and 32Dcl3 cells (G-CSF–responsive...
A

TWEARDY ET AL

A

BMMC

CD34+

Hep

O

G

GM

I

O

G

GM

I

SIF-A

SIF-B

SIF-C

B

Time (min)

0

1

2

5

15

30

60

120

SIF-A

DISCUSSION

G-CSF activated a DNA binding protein in human neutrophils, bone marrow cells, and G-CSF-responsive murine cell lines that bound hSIE duplex oligonucleotide, forming murine cell line) contained two distinct DNA-binding protein complexes, one of which is antigenically related to Stat3. As shown in Fig 4, Stat3 antibody supershifted the major portion of the G-SIF-A complex formed in samples containing extracts from G-CSF–stimulated AML-193 and 32Dc13 cells; however, a residual unshifted SIF-A band remained. As noted previously, G-CSF also activated a SIF-B complex in AML-193 cells. SIF-B complex formation was also observed in 32Dc13 cells. The composition of this complex remains to be determined; of note, however, its formation and mobility appeared to be unaffected by anti-Stat3 antisera.

The distal half of the cytosolic domain of the G-CSFR is essential for optimal activation of G-SIF-A. To map the cytosolic portion of the G-CSFR required for G-SIF-A activation, we examined the IL-3-dependent murine pro-B-cell line, BAF/BO3, transfected with full-length and truncated mutant forms of the G-CSFR (Fig 5A). The resultant stably transfected cell lines were designated B-183, B-96, B-57, and B-26. We have recently shown that G-CSFR cell surface expression in the transfected cell lines was greatest in B-57 > B-26 > B-96 > B-183, as determined using flow cytometry analysis using affinity-purified G-CSFR antibody. B-183, B-96, and B-57 proliferated well in response to G-CSF, whereas B-26 and the parental cell line did not proliferate at all (Fig 5B). Whole-cell extracts were prepared from each BAF cell line before and after stimulation with G-CSF or IFN-γ. Despite being able to proliferate in response to G-CSF, extracts of B-57 cells, like those of BAF/BO3 and B-26 cells, failed to activate an hSIE binding factor in response to G-CSF. In B-96, G-CSF-stimulated DNA-binding proteins that shifted hSIE, forming a weak G-SIF-A band and a SIF-B band. In contrast, G-CSF stimulation of B-183 cells strongly activated DNA binding proteins that shifted the hSIE to form a prominent G-SIF-A band and a weak SIF-B, a pattern similar to that observed in G-CSF–stimulated 32Dc13 cells (Fig 4A). The addition of Stat3 polyclonal antisera to samples containing extracts from G-CSF–stimulated B-183 and B-96 cells showed that, similar to 32Dc13 cells, the G-SIF-A band formed from B-183 and B-96 extracts was composed of both an hSIE/Stat3 complex and an hSIE/protein complex lacking Stat3.
a protein complex that migrated on EMSA with mobility similar to SIF-A (G-SIF-A). G-SIF-A activation in human neutrophils was detectable at a concentration of G-CSF as low as 1 ng/mL and occurred within 1 minute. Stimulation was maximal at 30 ng/mL and at 15 to 30 minutes. G-SIF-A translocated to the nucleus within 1 minute, with more activity residing in the nucleus than the cytoplasm by 30 minutes. G-SIF-A did not shift duplex oligonucleotides used to characterize Stat1, 2, 4, 5, or 6; nor could these oligonucleotides compete for hSIE binding. In addition, G-SIF-A in human neutrophils and bone marrow cells did not supershift to other STAT proteins, tyrosine phosphorylation of StatG is underway to distinguish between these alternatives.

Preliminary characterization, thus far, has shown that treatment of G-CSF–stimulated neutrophils with the tyrosine kinase inhibitor, genistein, as described, blocked StatG activation. In addition, treatment with pervanadate (vanadyl peroxide), which enhances tyrosine phosphorylation in whole cells, prolonged the activity of StatG (A. Chakraborty, unpublished data). These results suggest that, similar to other STAT proteins, tyrosine phosphorylation of StatG is essential for DNA binding.

Investigations involving Stat1, Stat3, and Stat6 suggest that they interact with the IFN-γ receptor, members of the IL-6 receptor family, and the IL-4 receptor, respectively, through binding of a phosphorylated tyrosine site on the receptor via their SH2 or SH3:SH2 domains. This interaction is proposed to cause juxtaposition of the STAT protein with a receptor-associated protein tyrosine kinase, resulting in phosphorylation of STAT on tyrosine and its subsequent dimerization. The cytosolic portion of the G-CSFR contains 4 tyrosine residues at amino acid positions 704, 729, 744, and 764, corresponding to cytosolic amino acid positions 73, 98, 113, and 133, respectively. (Fig 5A). BAF/B03 cells expressing truncated receptor mutants HGR-26 and HGR-57, which do not contain a tyrosine within their cytosolic domain, did not show G-SIF-A activation in response to G-CSF. In addition, in cells expressing HGR-96, which contains only one tyrosine (Y704) in its cytosolic domain, only modest activation of G-SIF-A was observed. These results suggest that Stat3 and the hSIE/protein complex lacking Stat3 (tentatively designated StatG) may be recruited preferentially to one of the distal 3 tyrosine sites (Y98, Y113, and Y133) within the cytosolic domain of the full-length receptor. This region of the G-CSFR cytosolic domain has been found to be critical for the induction of differentiation by G-CSF. The finding that Stat3 and possibly StatG are maximally activated by full-length receptor, together with the fact that their activation is not required for proliferation, suggests that they may be specifically involved in the signaling pathway directing myeloid differentiation by G-CSF.

The unique hSIE-binding factor activated in G-CSF–stimulated normal human myeloid cells, tentatively designated StatG, appears to be distinct from the six STAT proteins that have been characterized thus far. All six STAT proteins, as well as StatG, bind to DNA elements 20 to 30 nucleotides long containing an 8- to 10-nucleotide core motif starting with TT and ending with AA. However, among the different DNA elements described, there is considerable variation in the nucleotide sequences within and surrounding this core motif that most likely contributes to their being preferential bound by individual STAT proteins. In addition to DNA binding preferences, there is considerable variability in the mobility of the different STAT proteins on EMSA. StatG resembles Stat3 in DNA binding preference and mobility on EMSA; however, because it does not supershift with anti-Stat3 antibodies, StatG is distinct from Stat3. Furthermore, on the basis of DNA binding preference, mobility on EMSA, and lack of immunologic cross-reactivity, StatG also appears to be distinct from Stat1, 2, 4, 5, and 6. StatG may represent a novel member of the STAT protein family or, alternatively, may arise through unique posttranscriptional or posttranslational modifications of one of the other STAT gene products. Further characterization and purification of neutrophil StatG is underway to distinguish between these alternatives.

Fig 4. Supershift analysis of G-SIF-A in normal and leukemic human and murine myeloid cells. In (A) and (B), HepG2 cells (Hep) were incubated in PBS alone or in PBS containing IL-6 (1,000 U/mL; +) for 15 minutes. PMN, 32DC13 cells, BMMC, or CD34+ bone marrow cells (CD34+) were incubated in PBS alone (O) or in PBS containing G-CSF (1,000 ng/mL; +) for 15 minutes before whole-cell extraction. Extracts were preincubated with or without Stat3 rabbit antiserum, 1 µg or 3 µg (A; 1 or 3) or 1 µg (B; +), before EMSA with radiolabeled hSIE. The positions of the SIF-A, B, and C complexes and the supershifted SIF-A complex (SS) are indicated on the left.
Fig 5. Mapping of hSIE-binding protein activation using full-length and truncated mutants of the G-CSFR in BAF/BO3 cells. (A) is a schematic representation of the G-CSFR showing the location within the cytoplasmic domain of the truncations, tyrosines, and homology boxes. The numbering above the diagram starts with the N-terminus; the numbering beneath starts with the first cytosolic amino acid. The extracellular (EC) and transmembrane (TM) domains of the receptor are indicated. Boxes 1 and 2 are regions conserved among several members of the hematopoietin receptor family. Box 3A is a region of conserved homology within the gp130 family of receptors including the G-CSFR; its functional significance has not been established. Box 3B is a region of conserved homology within the gp130 family of receptors that appears to be necessary for activating acute-phase protein gene transcription. In (B), stably transfected cell lines (B-183, B-96, B-57, and B-26) and the parental cell line BAF/BO3 were examined for [3H]thymidine uptake in response to G-CSF as described in the Materials and Methods. CPM were normalized in each cell line to the response in 10% WEHI-3B supernatant (% IL-3 response). Data shown represent the mean of two experiments. In (C), the indicated cell lines were incubated in PBS alone (O), G-CSF (10 ng/mL; G), or IFN-γ (1,000 U/mL; I) for 15 minutes at 37°C. Whole-cell extracts were examined by EMSA using radiolabeled hSIE. The positions of the SIF-A, B, and C complexes and the supershifted SIF-A band are indicated to the left of the autoradiogram.
G-CSF ACTIVATION OF A DISTINCT STAT-LIKE PROTEIN

We previously identified a potential tyrosine-activated motif (TAM) within the C-terminal half of the cytosolic domain of the G-CSFR from cytosolic amino acids 99 to 117 that may serve as the Syk binding site. According to this model, Syk would be recruited to Y99 and Y117, leaving Y133 within the C-terminal half of the G-CSFR available for StatG binding. This binding would juxtapose StatG and Syk, allowing for the possibility that Syk could phosphorylate and thereby activate StatG.

Our findings in AML-193 suggest that StatG is activated in this cell line in response to G-CSF, in addition to Stat3, as previously reported. Stat3 was not activated in normal human myeloid cells, including CD34+ bone marrow cells, which suggests that its activation by G-CSF in human leukemic cells is aberrant. This finding raises the possibility that activation of alternative transcription factors by G-CSF in some leukemic cells may contribute to their failure to activate the normal differentiation program in response to this cytokine. The aberrant activation of Stat3 in some AML cells may be the result of its being abnormally expressed in these cells or may be secondary to its recruitment and activation by an abnormal G-CSFR isoform. Each of these possibilities is being actively investigated.

In contrast to G-CSF, GM-CSF did not activate SIF-A in normal human myeloid cells. Many studies have shown that, although there is some overlap, G-CSF and GM-CSF each have distinct biologic effects. Most notable is the ability of G-CSF to specifically drive the terminal differentiation of committed myeloid precursors to neutrophils. Cloning of their receptors has given substantial insight into their distinct biologic functions by showing that, although they both belong to the hematopoietin/cytokine receptor family, each belongs to a distinct subfamily of this family. We have previously delineated overlap in the signaling pathway for G-CSF and GM-CSF. Both were shown to activate Lyn and Syk, although Syk activation by G-CSF was more dramatic than that by GM-CSF. Our finding of G-SIF-A activation by G-CSF but not by GM-CSF shows a clear divergence in signaling and transcriptional pathways for these two hematopoietins in normal cells. Recent studies in murine myeloid cells showing that GM-CSF, along with IL-3 and IL-5, activates Stat5 support the conclusion that G-CSF and GM-CSF signaling diverge at the level of STAT protein activation.

The unique ability of G-CSF to drive terminal neutrophilic granulocyte differentiation together with our findings that G-SIF-A activation does not correlate with proliferation, is specific for G-CSF versus GM-CSF, and maps to the differentiation-specific region of the G-CSFR, implicates a critical role for G-SIF-A in the neutrophilic differentiation program.

ACKNOWLEDGMENT

We are indebted to Dr Suzanne Ildstad and members of her laboratory for providing the vertebral bone marrow cells and to Dr Hiroshi Wakao for providing the Stat5 antibody.

REFERENCES


From www.bloodjournal.org by guest on August 30, 2017. For personal use only.
of the family of signal transducers and activators of transcription. Proc Natl Acad Sci USA 91:4806, 1994
19. Waikao H, Gouilleux F, Groner B: Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. EMBO J 13:2182, 1994
Granulocyte colony-stimulating factor rapidly activates a distinct STAT-like protein in normal myeloid cells

DJ Tweardy, TM Wright, SF Ziegler, H Baumann, A Chakraborty, SM White, KF Dyer and KA Rubin