The Apoptosis-inducing Granulocyte-Macrophage Colony-stimulating Factor (GM-CSF) Analog E21R Functions through Specific Regions of the Heterodimeric GM-CSF Receptor and Requires Interleukin-1β-converting Enzyme-like Proteases

(Received for publication, August 7, 1996, and in revised form, December 30, 1996)

Per O. Iversen‡‡, Timothy R. Hercus‡, Betty Zacharakis‡, Joanna M. Woodcock‡, Frank C. Stomski‡, Sharad Kumar¶¶, Brad H. Nelson**t, Atsushi Miyajima‡‡, and Angel F. Lopez‡‡‡‡

From the Divisions of Human Immunology and Haematology, Hanson Centre for Cancer Research, IMVS, Adelaide, 5000 S.A., Australia; the Fred Hutchinson Cancer Research Center, Seattle, Washington 98104, and the Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113, Japan

The granulocyte-macrophage colony-stimulating factor (GM-CSF) analog E21R induces apoptosis of hemopoietic cells. We examined the GM-CSF receptor subunit requirements and the signaling molecules involved. Using Jurkat T cells transfected with the GM-CSF receptor we found that both receptor subunits were necessary for E21R-induced apoptosis. Specifically, the 16 membrane-proximal residues of the α subunit were sufficient for apoptosis. This sequence could be replaced by the corresponding sequence from the interleukin-2 receptor common γ subunit, identifying this as a conserved cytokine motif necessary for E21R-induced apoptosis. Cells expressing the α subunit and truncated βc mutants showed that the 96 membrane-proximal residues of βc were sufficient for apoptosis. E21R, in contrast to GM-CSF, did not alter tyrosine phosphorylation of βc, suggesting that receptor-associated tyrosine kinases were not activated. Consistent with this, E21R decreased the mitogen-activated protein kinase ERK (extracellular signal-regulated kinase). E21R-induced apoptosis was independent of Fas/APO-1 (CD95) and required interleukin-1β-converting enzyme (ICE)-like proteases. In contrast, Bcl-2, which protects cells from growth factor deprivation-induced cell death, did not prevent this apoptosis. These findings demonstrate the GM-CSF receptor and ICE-like protease requirements for E21R-induced apoptosis.

The multifunctional human cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) regulates the function and viability of a wide range of hemopoietic cells (for review, see Ref. 1). GM-CSF exerts its biological effects through binding to its high affinity, heterodimeric receptor complex composed of a low affinity GM-CSF-specific α subunit (GMR-α) and a common β subunit (βc) shared with the receptors for interleukin-3 (IL-3) and IL-5 (2). Amino acid residue 21 in the GM-CSF molecule is essential for binding to its GM-CSF receptor αβc complex (3). Substitution of glutamic acid for arginine at this position in GM-CSF resulted in a GM-CSF analog (E21R) that bound to the low affinity GMR-α, was devoid of high affinity binding to the GM-CSF receptor αβc complex, and effectively antagonized GM-CSF in binding experiments and functional assays (4). Furthermore, E21R induced apoptosis (programmed cell death) in hemopoietic cells expressing the GM-CSF receptor (5). Importantly, E21R directly induced apoptosis in the absence of any preexisting GM-CSF, and this appeared to be an active process since inhibition of protein phosphorylation, transcription, and protein synthesis rescued the cells from E21R-induced apoptosis (5). Moreover, E21R induced apoptosis even if the survival factors granulocyte colony-stimulating factor or stem cell factor were added to the cells. However, addition of IL-3 blocked E21R-induced apoptosis, indicating that ligand-mediated engagement of βc is central for maintaining cell viability (5). These results raised the question of the role played by each GM-CSF receptor subunit in E21R-induced apoptosis.

GMR-α is the major binding subunit of the GM-CSF receptor complex and plays a role in biological signaling. Mapping of the cytoplasmic domain of the GMR-α subunit using truncated receptor mutants suggested that the membrane-proximal region (upstream of residue 382) is important for growth of BaF3 cells (6–9). We wanted to study if the same regions were involved in E21R-induced apoptosis.

The βc alone does not bind GM-CSF, but confers high affinity binding of GM-CSF when coexpressed with the low affinity GMR-α subunit (10). Moreover, βc is crucial for signal transduction generated by the GM-CSF receptor (10, 11). Studies of βc mutants with cytoplasmic truncations coexpressed with wild-type GMR-α in BaF3 cells revealed two regions critical for signaling. The membrane-proximal region (upstream of residue 517) is involved in phosphorylation, proliferation, and viability (12–14). The distal region (between residues 626 and 763) is essential for activation of signaling proteins including ras and mitogen-activated protein (MAP) kinases (14) and rescue from growth factor deprivation-induced apoptosis (15). Although βc is essential for survival, its role in E21R-induced apoptosis is not known. The absence of high affinity binding of E21R (presumably the result of a lack of βc contact) suggested that
binding to GMR-α was sufficient and that βc was not required for E21R-induced apoptosis.

Ligand binding of the GM-CSF receptor αβc complex leads to phosphorylation of βc, the Jak2 kinase, and MAP kinases (8, 14, 16). Certain stimuli such as activation of Fas/APO-1 (CD95), osmotic shock, and UV irradiation cause an increase in the activity of the MAP kinase c-Jun-NH2-terminal kinase (JNK) compared with the extracellular signal-regulated kinase (ERK), and this imbalance has been associated with apoptosis (17–21). As GM-CSF can activate the MAP-kinases, it was of interest to see if activation of JNK and ERK is associated with E21R-induced apoptosis.

Although various external stimuli can initiate apoptosis through different signaling cascades, some of these converge at the level of one or several interleukin-1β-converting enzyme (ICE) and related cysteine proteases (for review, see Ref. 22). It has not been established whether ICE and ICE-like proteases are involved in E21R-induced apoptosis.

In this study we sought to determine (i) the requirements of the GMR-α and β subunits in E21R-induced apoptosis; (ii) the role played by JNK and ERK; (iii) whether E21R activates the Fas/APO-1 (CD95) receptor; and (iv) if ICE-like proteases are essential for E21R-induced apoptosis. We show that the membrane-proximal regions of both GMR-α and βc are required for E21R-induced apoptosis. This process does not involve tyrosine phosphorylation of βc, but treatment with E21R alters the ERK/JNK balance. The apoptosis-inducing surface receptor Fas/APO-1 (CD95) is not activated with E21R. Furthermore, we provide evidence for involvement of ICE-like proteases following E21R treatment. Finally, the physiological cell death inhibitor Bcl-2 does not inhibit apoptosis by E21R, indicating that the molecular mechanism of this pathway is distinct from that induced by growth factor deprivation.

Materials and Methods

Permission was approved by ethics committees to collect blood from leukemic patients.

Cells—Peripheral blood was collected from patients with untreated adult acute myeloid leukemia (AML). Primary blast cells expressing functional heterodimeric GM-CSF receptors were isolated and cultured as described previously (5). These AML cells did not express mRNA for acute myeloid leukemia (AML). Primary blast cells expressing functional heterodimeric GM-CSF receptors were isolated and cultured as described previously (5). These AML cells did not express mRNA for GM-CSF, and they were devoid of endogenous GM-CSF production (5). We also studied Jurkat T cells. This human cell line grows autonomously and was maintained in RPMI medium supplemented with 10% fetal calf serum without exogenous growth factors. Nonstimulated Jurkat T cells do not produce detectable GM-CSF mRNA or protein.

Transfection of Jurkat T Cells—DNA constructs were introduced into Jurkat T cells by electroporation using a Gene Pulser (Bio-Rad, North Ryde, NSW, Australia). We cotransfected 2 × 107 cells in 0.3 ml of RPMI medium with 3 μg of GMR-α constructs, 7 μg of βc constructs, and 10 μg of apoptosis inhibitor expression constructs, and at 960 g of GMR-α constructs, 7 μg of βc constructs, and 10 μg of apoptosis inhibitor expression constructs, and at 960 microfrads and 270 V.

The generation of the cDNAs and the corresponding vectors for the GMR-α and βc mutants have been described elsewhere (7, 8, 23, 24). These receptor cDNA constructs were transiently transfected into the Jurkat T cells before they were sorted and studied after 48 h.

Plasmids encoding RSV/Bcl-2, pCXN2/CrmA, or pcDNA3/p35 were introduced into Jurkat T cells, and stable clones were selected for resistance to neomycin (1.5 mg/ml, CrmA and p35) or hygromycin (0.5 mg/ml, Bcl-2). After selection for antibiotic resistance over the next 15 days, we transiently transfected the cells with both GMR-α and βc cDNA as outlined above.

Immunoprecipitation and Western Blotting—The methods for immunoprecipitation of the βc subunit and Western blotting of phosphorylated βc have been detailed recently (25). Briefly, AML cells (107/sample) were stimulated with either GM-CSF (10 ng/ml, Genetech Institute, Cambridge, MA) or E21R (1 μg/ml, BresaGen, Adelaide, Australia) for selected time periods before they were lysed, and βc was immunoprecipitated with our anti-βc monoclonal antibody 4F3. Immunoprecipitated βc was then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions.

In separate experiments, AML cells were metabolically labeled with 32P-O4 (1 μCi/107 cells, BresaGen) overnight prior to cytokine stimulation. Gels with immunoprecipitated βc were analyzed with a Phospho-Imager (Molecular Dynamics, Sunnyvale, CA).

In other experiments we specifically examined phosphorylation of immunoprecipitated βc using an anti-phosphotyrosine monoclonal antibody (3-365-10, Bio-Rad, Hercules, CA) and the ECL detection kit (Amersham, Little Chalfont, U. K.).

Analysis of MAP Kinase Activity—To measure the JNK/ERK activity we used a kinase method described previously (26). Essentially, AML cells (107/sample) were cultured with either GM-CSF (10 ng/ml), E21R (10 μg/ml), or subjected to 30 min of osmotic stress by propositol (0.6 g, Sigma, Castle Hill, NSW, Australia). The cells were washed, and the supernatant was immunoprecipitated with either anti-JNK1 or an anti-ERK1 monoclonal antibody (1 μg/ml, Pharmigen, San Diego, CA). Immunoprecipitates were then resuspended in kinase buffer supplemented with 20 μCi of [γ-32P]ATP (BresaGen) and 20 μg of either the fusion protein c-Jun (1–165)-GST (Upstate Biotechnology, Inc., Lake Placid, NY) or myelin basic protein (Sigma). The kinase reaction was performed for 30 min at 37 °C before it was terminated with an equal volume of Laemmli sample buffer. The phosphorylation of products was examined after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7.5%) using the PhosphoImager.

Compounds—To test whether the Fas/APO-1 (CD95) or tumor necrosis factor (TNF) receptors were activated upon adding E21R, we cultured AML cells for 48 h with E21R (1 μg/ml) with or without a soluble Fas receptor (anti-Fas, 20 μg/ml) or a soluble TFN receptor (TNFR, 20 μg/ml) (27). As positive controls we measured, after 24 h, either the viability of Jurkat T cells cultured with an anti-CD3 monoclonal antibody (OKT3, 100 μg/ml) or without anti-Fas (20 μg/ml), or the viability of the human laryngeal carcinoma cell line HeP2 (28) cultured with TNFα (50 ng/ml) with or without anti-TNFβ (20 μg/ml).

We used the ICE-inhibitor Tyr-Val-Ala-Asp-chloromethylketone (YVAD-CMK; Bachem, Switzerland) or the alkylating agent iodoacetamide (Sigma) to block protease activity. Dexamethasone (0.1 mm) (Sigma) was used to induce apoptosis of Jurkat T cells with or without overexpression of Bcl-2.

Determination of Apoptosis—The degradation of chromosomal DNA into low molecular weight fragments was displayed on 1.2% agarose gels after an overnight incubation with lysis buffer followed by extraction with organic solutions as described elsewhere (29).

We quantitated the number of apoptotic cells by either measuring the reduced binding of propidium iodide to DNA using an EPICS-Profile II Flow Cytometer (Coulter Electronics, Hialeah, FL) as outlined (30) or by determination of trypan blue exclusion.

Results

The Membrane-Proximal Regions of the Cytoplasmic Domains of Both GM-R-α and β Are Required for E21R-Induced Apoptosis—All human, primary, hemopoietic cells express both the GMR-α and βc. To establish the individual requirements of GMR-α and βc for E21R-induced apoptosis, we expressed either or both receptor subunits in Jurkat T cells since these cells do not have endogenous expression of the GM-CSF receptor. Using monoclonal antibodies and flow cytometry we isolated the GM-CSF receptor-positive cells and confirmed the expression of the GMR-α or the βc mutants and that the levels of expression were similar. Furthermore, the expression levels remained constant over the next 40 h (data not shown). E21R (10 μg/ml) induced apoptosis only in cells expressing both receptor subunits and in a dose-dependent manner (Fig. 1A) with a maximal effect seen after 30 h (Fig. 1B). Cells expressing either receptor subunit alone remained fully viable. We identified key features of apoptosis (22) such as condensation of the chromatin and decreases in size of the nuclei and cells upon addition of E21R by examination of electron micrographs (Fig. 2, A and B) and demonstration of fragmented DNA (Fig. 2C).

Since Jurkat T cells grow in a GM-CSF-independent manner these experiments further illustrate the active nature of the E21R-induced apoptosis.

To study in more detail the cytoplasmic regions of the GMR-α and βc involved in E21R-induced apoptosis, we expressed a...
series of mutant receptors in Jurkat T cells (Fig. 3). In cells expressing wild-type GMR-α and β mutants having up to 255 amino acids deleted (β344, β345), E21R (10 μg/ml) induced apoptosis in a manner similar to cells expressing the wild-type GM-CSF receptor complex (Fig. 4). Cells with mutant β344 showed intermediate decline in viability, whereas cells carrying the βα17 and βα45 mutants were resistant to E21R treatment. Addition of wild-type GM-CSF alone had no impact on the viability of these cells (data not shown). Notably, the chimeric receptor composed of the extracellular domain of β and the full-length cytoplasmic domain of the IL-2 receptor β subunit was unable to signal apoptosis in response to E21R (Fig. 4). Thus, the membrane-proximal region of the cytoplasmic domain of β appears important for E21R-induced apoptosis.

With a similar approach using cells expressing wild-type βc and truncated GMR-α mutants (Fig. 3), we determined the cytoplasmic region of the GMR-α subunit required for E21R-induced apoptosis. Fig. 5A shows that a region spanning 16 amino acids downstream of the transmembrane domain was necessary for E21R (10 μg/ml) to induce apoptosis. Interestingly, a GMR-α/IL-2 receptor common γ subunit chimera allowed E21R-induced apoptosis, indicating that the cytoplasmic region of the IL-2 receptor common γ subunit can substitute for GMR-α in this effect (Fig. 5B). Consistent with the need for the membrane-proximal region of the receptor, the GMR-α/CPRox chimera prevented E21R-induced apoptosis (Fig. 5B). Addition of wild-type GM-CSF alone had no impact on the viability of the cells (data not shown).

E21R Induces Apoptosis Independent of Phosphorylation of βc—We next examined whether phosphorylation of βc accompanied E21R-induced apoptosis. We could not detect any alteration in the level of total phosphorylation of βc immunoprecipitated from AML cells metabolically labeled with 32P and treated with E21R (1 μg/ml; Fig. 6A). GM-CSF (10 ng/ml), on the other hand, markedly enhanced phosphorylation of βc. This observation was confirmed and extended with immunoprecipitated βc and an anti-phosphotyrosine immunoblot. Whereas GM-CSF caused a rapid and clear increase in tyrosine phosphorylation of βc, E21R did not (Fig. 6B). Tyrosine phosphorylation of βc is therefore apparently not essential for E21R-induced apoptosis. Neither did we observe any detectable phosphorylation of the GMR-α subunit upon addition of E21R (data not shown). Moreover, in separate communoprecipitation experiments using anti-βc antibody for immunoprecipitation and anti-JAK2 antibody for Western blotting, we noted that JAK2 was intrinsically associated with βc and that neither GM-CSF nor E21R altered the levels of JAK2 preassociation up to the 30-min time point. Furthermore, GM-CSF, but not E21R, induced phosphorylation of JAK2 (data not shown).

E21R Suppresses ERK Activity without Affecting JNK Activity—To assess whether MAP kinases are associated with E21R-induced apoptosis, we measured the activities of immunoprecipitated ERK1 and JNK1 in a kinase assay. It is evident from Fig. 7 that E21R (1 μg/ml) prominently decreased ERK1 activity without affecting JNK1 activity in AML cells. These data reflect the actual activity of the MAP kinases and not their concentrations since we could not detect any alteration in the total amount of either ERK1 or JNK using immunoprecipitation followed by Western blotting (data not shown).

E21R Induces Apoptosis Independent of the Fas/APO-1 (CD95) or TNF Receptors—We studied E21R-induced apoptosis of AML cells cultured with soluble Fas and soluble TNF receptors (Fig. 8A). These soluble receptors did not affect the decline in AML cell viability upon addition of E21R (1 μg/ml), whereas anti-Fas inhibited Jurkat T cells from dying following activation of the Fas/APO-1 (CD95) system with an anti-CD3 monoclonal antibody, and anti-TNFR inhibited TNF-α-induced apoptosis of Hep2 cells (Fig. 8B).

E21R-induced Apoptosis Is Dependent on ICE-like Proteases—ICE-like proteases participate in the induction of cell death in response to several different stimuli (22, 31). Apoptosis mediated by several members of the family of ICE-like proteases has been shown to be inhibited by the cysteine protease inhibitor iodoacetamide and the ICE-inhibitor YVAD-CMK and the viral proteins CrmA and p35 (for review, see Ref. 22). We used these inhibitors to test whether E21R induced
apoptosis via ICE-like proteases. Indeed, addition of iodoacetamide or YVAD-CMK dose dependently blocked E21R-induced apoptosis in Jurkat T cells (Fig. 9A) and in AML cells (data not shown). Treatment with YVAD-CMK or iodoacetamide alone had no impact on cell viability (Fig. 9A). Jurkat T cells overexpressing either CrmA or p35 and the wild-type GM-CSF αβ receptor complex were rescued from E21R-induced apoptosis (Fig. 9B). However, overexpression of the oncogene product Bcl-2, an apoptosis-inhibitor (32), failed to block E21R-induced apoptosis, although it prevented dexamethasone-induced apoptosis of these cells (Fig. 9C).

**DISCUSSION**

We show here that apoptosis induced by the GM-CSF analog E21R requires the coexpression of GM-Rα and β cells on the cells. Data obtained with the truncated GM-Rα mutants revealed that a short membrane-proximal region was crucial for E21R to induce apoptosis. This notion was strengthened further with data obtained from chimeric GM-Rα receptor constructs containing either full-length or regions of the cytoplasmic IL-2 receptor γ common subunit. Interestingly, the membrane-proximal region of the cytoplasmic domain of GM-Rα contains a proline-rich motif termed box 1 which is conserved among the IL-3Rα and IL-5Rα subunits, and the common IL-2 receptor γ subunit (24). Deletions within this region in the human GM-Rα, and in both the human and murine IL-5Rα, abolished proliferation and protein phosphorylation (7, 33, 34), demonstrating its pivotal role in cell growth regulation. Our data pose the intriguing and novel possibility that this region might also be involved in eliciting a death signal under certain conditions.

Using a series of truncated βc mutants, we found that a membrane-proximal region spanning approximately 100 amino
acids of the cytoplasmic domain is sufficient for E21R-induced apoptosis. This particular region has been shown previously to be important for proliferation of BaF3 cells (8), but it does not include the more distal region involved in promoting cell survival (15). Moreover, E21R-induced apoptosis specifically required this membrane-proximal region of βc since the chimeric receptor containing full-length cytoplasmic domain of the IL-2 receptor β subunit, whose amino acid sequence shares no significant homology with βc, failed to signal apoptosis in response to E21R.

Stimulation with GM-CSF results in phosphorylation of both βc and the associated JAK2 kinase (8, 16), and tyrosine phosphorylation of the more distal residue 750 of βc is important for maintaining viability of BaF3 cells (12). Whereas GM-CSF induced a rapid increase in both total phosphorylation and tyrosine phosphorylation of βc, E21R was without any effect. Consistent with this finding is the observation that E21R, in contrast to GM-CSF, did not activate JAK2. Apparently, βc propagates an E21R-initiated death signal at least without requiring βc tyrosine phosphorylation, which is consistent with our previous observation that the tyrosine kinase inhibitor genistein did not prevent E21R-induced apoptosis (5).

An increase in the activity of the MAP kinase JNK concomitant with a decreased ERK activity has been observed in various cells undergoing apoptosis (17–21). A novel finding in the present study is that E21R-induced apoptosis leads to an increased ratio of JNK to ERK activity because of a decrease in...
Among the apoptosis-inducing events most thoroughly studied are the Fas-related apoptosis and cell death caused by a lack of growth factors (26, 32, 36). Our data indicate that the apoptotic pathway triggered by E21R binding to the GM-CSF receptor αβ complex is probably different from Fas involvement. First, we could not detect surface expression of the Fas ligand on primary AML cells (data not shown). Second, a soluble Fas receptor did not prevent E21R-induced apoptosis. Third, E21R did not affect the expression and activation of the serine/threonine kinase FAST and the nuclear RNA-binding protein TIA1 (data not shown) involved in Fas-mediated apoptosis of Jurkat T cells (36). Fourth, the cytoplasmic domains of GMR-α and βc do not possess any significant homology to the death domains identified in the Fas/APO1 and TNF receptors (37, 38).

Although Bcl-2 can prevent apoptosis following growth factor deprivation in various cell types (32, 39), the data presented here indicate that the mechanism of E21R-induced apoptosis is distinct from growth factor deprivation. This is based on the inability of overexpression of Bcl-2 to rescue Jurkat T cells (Fig. 9B) or primary AML cells (data not shown) treated with E21R despite being able to rescue Jurkat T cells from dexamethasone-induced apoptosis (Fig. 9C). In addition, the protein kinase C inhibitor staurosporine blocked E21R-induced apoptosis of AML cells (5), but it failed to suppress death caused by growth factor deprivation (data not shown). Collectively, our data suggest that the death signal initiated with E21R ultimately leads to activation of one or more ICE-like cysteine proteases, and in a Fas/APO1 (CD95)/Bcl-2-independent manner.

We suggest that a tyrosine-dephosphorylated state of βc might be important for E21R-induced apoptosis. Possibly E21R disrupts a preformed, productive GM-CSF receptor (5), and events downstream of the receptor might alter the balance among the activity of the MAP kinases JNK and ERK leading to gene expression involved in triggering apoptosis, such as activators of ICE-like proteases. Future studies are required to define the exact mechanism by which the GM-CSF receptor elicits the death signal within the cell and the identity of any resulting gene products.

Acknowledgments—We thank Dr. A. Strasser for comments on the manuscript. The cDNA for the truncated GMR-α mutants was from A. Kraft. The cDNA for Bcl-2 was from Dr. D. Vaux, CrmA from Dr. D. Pickup, and p35 from Dr. V. Dixit. The supplies of GM-CSF were from Genetics Institute, E21R from BresaGen, and soluble Fas and TNF receptors from Dr. P. Krammer.

REFERENCES

1. Metcalf, D. (1984) The Hemopoietic Colony-stimulating Factors. Elsevier, Amsterdam.

2. Lopez, A. F., Elliott, M. J., Woodcock, J., and Vadas, M. A. (1992) Immunol. Today 13, 495–500.

3. Hercus, T. R., Cambareri, B., Dottore, M., Woodcock, J. M., Bagley, C. J., Vadas, M. A., Shannon, M. F., and Lopez, A. F. (1994) Blood 83, 3505–3508.

4. Hercus, T. R., Bagley, C. J., Cambareri, B., Dottore, M., Woodcock, J. M., Vadas, M. A., Shannon, M. F., and Lopez, A. F. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5838–5842.

5. Ivesen, P. O., To, L. B., and Lopez, A. F. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2785–2789.

6. Muto, A., Watanabe, S., Itoh, T., Miyajima, A., Yokota, T., and Ariai, K.-I. (1995) J. Allergy Clin. Immunol. 96, 1100–1114.

7. Poletsky, A., Zhao, Y., Libly, M. B., and Kraft, A. S. (1994) J. Biol. Chem. 269, 14697–14631.

8. Sakamaki, K., Miyajima, I., Kitamura, T., and Miyajima, A. (1992) EMBO J. 11, 3541–3549.

9. Weiss, M., Yokoyama, C., Shikama, Y., Naugle, C., Druker, C., and Sieff, S. A. (1993) Blood 82, 3298–3306.

10. Kitamura, T., Hayashida, K., Sakamaki, K., Yokota, T., Ariai, K.-I., and Miyajima, A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5028–5032.

11. Watanabe, S., Mui, A. L. F., Muto, A., Chen, J. X., Hayashida, K., Yokota, T., Miyajima, A., and Ariai, K.-I. (1993) Mol. Cell. Biol. 13, 1440–1448.

12. Inhorn, R. C., Carless, N., Duretin, M., Frank, D. A., and Griffin, J. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8665–8669.

13. Itoh, T., Muto, M., Watanabe, S., Miyajima, A., Yokota, T., and Ariai, K.-I. (1996) J. Biol. Chem. 271, 7587–7592.
14. Sato, N., Sakamaki, K., Terada, N., Arai, K.-I., and Miyajima, A. (1993) *EMBO J.* **12**, 4181–4189
15. Kinoshita, T., Yokota, T., Arai, K.-I., and Miyajima, A. (1995) *EMBO J.* **14**, 266–275
16. Quelle, F. W., Sato, N., Witthuhn, B. A., Inhorn, R. C., Eder, M., Miyajima, A., Griffin, J. D., and Bile, J. N. (1994) *Mol. Cell. Biol.* **14**, 4335–4341
17. Cano, E., and Mahadevan, L. C. (1995) *Trends Biochem. Sci.* **20**, 117–122
18. Chen, Y.-R., Meyer, C. F., and Tan, T.-H. (1996) *J. Biol. Chem.* **271**, 631–634
19. Verheij, M., Bose, R., Lin, X. H., Yao, B., Jarvis, W. D., Grant, S., Birrer, M. J., Szabo, E., Zon, L. I., Haimovitz-Friedman, A., Fuke, Z., and Kolesnick, R. N. (1996) *Nature* **380**, 75–79
20. Wilson, D. J., Fortt, K. A., Lynch, D. H., Mattingly, R. R., Macara, I. G., Posada, J. A., and Budd, R. C. (1996) *Eur. J. Immunol.* **26**, 989–994
21. Nelson, B. H., Lord, J. D., and Greenberg, P. D. (1994) *Nature* **369**, 333–336
22. Nelson, B. H., Lord, J. D., and Greenberg, P. D. (1996) *Mol. Cell. Biol.* **16**, 309–317
23. Patel, T., Gores, G. J., and Kaufmann, S. H. (1996) *FASEB J.* **10**, 587–597
24. Chinnaiyan, A. M., Orth, K., O’Rourke, K., Duan, H., Poirier, G. G., and Dixit, V. M. (1996) *J. Biol. Chem.* **271**, 4573–4576
25. Vaux, D. L., Cory, S., and Adams, J. M. (1988) *Nature* **335**, 440–442
The Apoptosis-inducing Granulocyte-Macrophage Colony-stimulating Factor (GM-CSF) Analog E21R Functions through Specific Regions of the Heterodimeric GM-CSF Receptor and Requires Interleukin-1β-converting Enzyme-like Proteases

Per O. Iversen, Timothy R. Hercus, Betty Zacharakis, Joanna M. Woodcock, Frank C. Stomski, Sharad Kumar, Brad H. Nelson, Atsushi Miyajima and Angel F. Lopez

J. Biol. Chem. 1997, 272:9877-9883.
doi: 10.1074/jbc.272.15.9877

Access the most updated version of this article at http://www.jbc.org/content/272/15/9877

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 37 references, 19 of which can be accessed free at http://www.jbc.org/content/272/15/9877.full.html#ref-list-1