Activation of the Jak2-Stat5 Signaling Pathway in Nb2 Lymphoma Cells by an Anti-apoptotic Agent, Aurintricarboxylic Acid*

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Biological effects of many hormones and cytokines are mediated through receptor-associated Jak tyrosine kinases and cytoplasmic Stat transcription factors, including critical physiological processes such as immunity, reproduction, and cell growth and differentiation. Pharmaceuticals that control Jak-Stat pathways are therefore of considerable interest. Here we demonstrate that a single Jak-Stat pathway can be activated by aurintricarboxylic acid (ATA), a negatively charged triphenylmethane derivative (475 Da) with anti-apoptotic properties. In prolactin (PRL)-dependent Nb2 lymphocytes, ATA sustains cell growth in the absence of hormone and mimicked rapid PRL-induced tyrosine phosphorylation of Jak2 and activation of Stat5a and Stat5b with tyrosine phosphorylation, heterodimerization, DNA binding, and induction of the Stat5-regulated pim-1 protooncogene. ATA also mimicked PRL activation of serine kinases ERK1 and ERK2. However, unlike PRL, ATA did not regulate Stat1 or Stat3. ATA also did not affect Jak3, which is activated in these cells by interleukin-2 family cytokines. Although the mechanism and specificity by which ATA activates Jak2, Stat5a, and ERKs in Nb2 cells are still unclear, the present study demonstrates that certain hormone or cytokine effects on Jak-Stat pathways can be discretely imitated by a low molecular weight, non-peptide pharmaceutical. The results are also consistent with Stat5 involvement in lymphocyte growth and survival.

Janus tyrosine kinases (Jaks) and cytoplasmic signal transducers and activators of transcription (Stats) are critical mediators for key biological effects of a number of polypeptide hormones and cytokines (1, 2). The regulatory functions of Jak-Stat signal pathways are still being widely investigated, and their pharmacological control is of significant clinical interest. In humans, genetic inactivation of Jak3 is associated with a severe combined immunodeficiency syndrome due to disruption of signals by many lymphocyte growth factors using the common interleukin-2 (IL2)1 receptor-γ (3). Furthermore, Stat1 deficiency is associated with severe disturbances in interferon-mediated immune responses (4), whereas Stat5a-deficient mice fail to establish prolactin (PRL)-induced milk production (5).

Recently, progress has been made with generating selective Jak2 inhibitors (6), and efforts to develop specific Jak3 inhibitors for use as immunosuppressive agents are ongoing. Conceptually, selective pharmacological activators of Jaks and Stats also have important therapeutic potential. Aurintricarboxylic acid (ATA) is a polyamionic aromatic compound with reported anti-apoptotic effects in several cell types, including Nb2 lymphocytes (7, 8). In Nb2 lymphocytes PRL stimulates Jak2 and cell proliferation (9, 10), whereas IL2, IL7, and IL9 stimulate Nb2 cell proliferation via Jak3 activation (11, 12). We now report that ATA treatment of Nb2 lymphocytes leads to rapid activation of Jak2 but not of other Jaks. ATA-induced Jak2 activation was associated with activation of transcription factors Stat5a and Stat5b in addition to serine kinases ERK1/2. However, unlike prolactin, ATA did not induce additional Stat1 and Stat3 signals. The present study describes activation of a Jak2-Stat5 pathway by ATA in Nb2 cells and suggests a novel pharmaceutical approach involving low molecular weight, non-peptide compounds to activate individual Jak-Stat pathways.

EXPERIMENTAL PROCEDURES

Materials—Ovine PRL (NIDDK-oPRL-19, AFIP-9221A) was supplied by the National Hormone and Pituitary Program, NIDDK, the National Institute of Child Health and Human Development, and the U.S. Department of Agriculture. Monoclonal antiphosphotyrosine antibody 4G10 and polyclonal rabbit antisera to-Jak1, Jak2, Tyk2, and Jak3 were purchased from Upstate Biotechnology Inc. (catalog numbers 06-321, 06-272, 06-255, 06-375, and 06-342, respectively). Polyclonal rabbit antisera specific to peptides corresponding to the unique COOH termini of Stat1a, Stat2, Stat3, Stat5a, and Stat5b were generated as described previously (13). Monoclonal antibodies for immunoblotting of Stat1 and Stat3 were obtained from Transduction Laboratories (catalog numbers S21120 and S21320, respectively). Rabbit antibodies to active MAPK were purchased from Promega (catalog number V667A), and a mouse monoclonal anti-panERK antibody was obtained from Transduction Laboratories (catalog number E11720/L3).

Cell Culture and Treatment—PRL-dependent Nb2 cells were originally developed by Dr. Peter Gout (14); the Nb2-SP clone also used in this work was provided by Dr. Henry Friesen (University of Manitoba, Winnipeg, Canada). Cells were grown in RPMI 1640 medium (Mediatech, catalog number 15-040-LM) containing 10% fetal calf serum (Intragen, catalog number 1020-90), 2 mm L-glutamine, 5 mM HEPES, pH 7.3, and penicillin-streptomycin (50 IU/ml and 50 μg/ml, respectively) at 37 °C with 5% CO2. Nb2 cells at a density of 1–1.5×106/ml were incubated for 20–24 h in lactogen-free RPMI 1640 medium, which instead of 10% fetal calf serum contained 1% gelatin horse serum (Sigma, catalog number H-1895).

Cell Proliferation Assay—Serum-deprived cells were dispensed into 96-well microtiter plates at 4×104 cells/100 μl and exposed to control medium, oPRL, or ATA as indicated for 48 h. Viable cells were measured metabolically by the Promega cell proliferation assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) as described previously (15). Colorimetric analysis was performed using a semiautomated plate reader from Dynatech Laboratories (MR9600) at absorbance 570 nm.

DNA Fragmentation Assay—DNA extracts were prepared from samples of 5×106 cells as described (16), treated with RNase (25 μg/ml),
and separated by 1.2% agarose gel electrophoresis. Gels were stained with ethidium bromide (0.5 μg/ml) and photographed using a UV transilluminator (Fotodyne Inc.).

Solubilization of Proteins, Immunoprecipitation, and Immunoblotting—Frozen pellets from 1 × 10⁶ Nb2 cells were thawed on ice and solubilized in 1 ml of lysis buffer containing 10 mM Tris-HCl, pH 7.6, 5 mM EDTA, 50 mM NaCl, 30 mM sodium pyrophosphate, 50 mM sodium fluoride, 1 mM sodium orthovanadate, 1% Triton X-100, 1 mM PMSF, 5 μg/ml aprotinin, 1 μg/ml pepstatin A, and 2 μg/ml leupeptin. Cell lysates were rotated at 4 °C for 60 min, and insoluble material was pelleted at 12,000 g for 30 min at 4 °C. Clarified lysates were incubated on ice for 20 min and then clarified by centrifugation at 12,000 g for 30 min at 4 °C. Clarified lysates were incubated for 60 min with protein A-Sepharose beads (Pharmacia Biotech Inc., catalog number 17-0780-01), and proteins were analyzed by SDS-PAGE and immunoblotting as described previously (17) using polyvinylidene difluoride membranes (Millipore, catalog number IPVH 00010) and horseshadish-conjugated secondary antibodies in conjunction with enhanced chemiluminescence (Millipore, catalog number 1PVH 00010) and horseradish-conjugated secondary antibodies in conjunction with enhanced chemiluminescence (Millipore, catalog number 1PVH 00010) and horseradish-conjugated secondary antibodies in conjunction with enhanced chemiluminescence (Millipore, catalog number 1PVH 00010) and horseradish-conjugated secondary antibodies in conjunction with enhanced chemiluminescence (Millipore, catalog number 1PVH 00010) and horseradish-conjugated secondary antibodies in conjunction with enhanced chemiluminescence (Millipore, catalog number 1PVH 00010) and horseradish-conjugated secondary antibodies in conjunction with enhanced chemiluminescence.

MAPK Assay—Quiescent Nb2 cells were stimulated with 100 nM PRL for 0–60 min at 37 °C and assayed at 4 °C in a buffer containing 20 mM HEPES, pH 7.5, 10 mM EGTA, 40 mM NaF, 20 μM aprotinin, 1 μg/ml pepstatin A, and 2 μg/ml leupeptin. Lysates were clarified by centrifugation at 15,000 x g for 20 min at 4 °C, and supernatants were incubated with 2 μg of monoclonal MAPK antibody 12C5 (Babeo, Inc.) for 1 h at 4 °C. Immunocomplexes were recovered using protein G-Sepharose beads (Pharmacia). After washing of immunoprecipitates three times with phosphate-buffered saline containing 1% Nonidet P-40, 2 mM vanadate, 100 mM Tris-HCl, pH 7.5, and 0.5% LiCl, 20 μl of kinase buffer was added (20 mM HEPES, pH 7.5, 12.5 mM α-glycerophosphate, 7.5 mM MgCl₂, 0.5 mM EGTA, 0.5 mM sodium fluoride, 0.5 mM sodium vanadate). The MAPK activity was analyzed by adding 30 μCi of [α-32P]ATP (20 Ci/mmol), 20 μM unlabeled ATP, and 1 μg of myelin basic protein/reaction as a substrate. After incubation at room temperature for 20 min, the reaction was terminated by addition of 6 μl of 4% sample buffer. Samples were heated at 95°C for 5 min and analyzed by SDS-PAGE, followed by autoradiography.

EMSA—Quiescent Nb2 cells were treated with or without PRL (10 nM) for 10 min, pelleted by centrifugation, and immediately solubilized in EMSA lysis buffer (20 mM HEPES, pH 7.0, 10 mM KCl, 1 mM MgCl₂, 20% glycerol, 0.2% Nonidet P-40, 1 mM dithiothreitol, 2 mM orthovanadate, 1 mM PMSF, 20 μg/ml aprotinin, and 20 μg/ml leupeptin. Lysates were clarified by centrifugation at 20,000 × g for 20 min at 4 °C. For the EMSA, 1 ng of oligonucleotide corresponding to the PRL response element (5′-gagcagtgtcagaggtaggtttttgctagag-3′) of the rat β-casein gene that had been end-labeled using polynucleotide kinase and [γ-32P]ATP was incubated with 1 μg of poly(dI-dC), 10 μg of protein from cellular lysates in a total of 40 μl of binding mixture (10 mM Tris-Cl, pH 7.4, 5 mM MgCl₂, 1 mM dithiothreitol, 10% glycerol) at room temperature for 20 min with preincubation of samples with 1 μl of either normal rabbit serum or antisera specific to Stat transcription factors as indicated. Polyacrylamide gels (5%) containing 5% glycerol and 0.25 × TBE (89 mM Tris base, 89 mM boric acid, 2 mM EDTA) were prerun in 0.25 × TBE buffer at 4–10 °C for 1.5 h at 270 V. Gels were run at room temperature for approximately 3 h at 250 V, dried by heating under vacuum, and exposed to x-ray film (X-Omat, Kodak).

Northern Blot Analysis—Nb2 cells treated with PRL or ATA were lysed using Trizol (Ambion) and processed as described previously (15). For hybridization a 2.7-kb insert containing a full-length human pim-1 cDNA cloned into the NcoI site of pBRK was used (provided by Dr. Nancy Magnuson, Washington State University, Pullman, WA).

RESULTS AND DISCUSSION

ATA Stimulates Nb2 Lymphocyte Growth and Protects against Apoptosis—The biological activity of ATA on Nb2 cells was tested using assays for proliferation and DNA fragmentation (Fig. 1). Exponentially growing, PRL-dependent Nb2 cells were serum-deprived for 24 h in PRL-free medium containing 1% horse serum and cultured with varying concentrations of ATA and varying concentrations of PRL for another 48 h. Cell growth was assayed using the MTT method as described. ATA alone stimulated Nb2 cell growth in a concentration-dependent manner, reaching peak levels at 100 μM, whereas PRL-induced cell growth leveled off at 16 ps (Fig. 1A). In combination, submaximal concentrations of ATA and PRL exerted additive effects on cell proliferation, but there was no synergy or additive effect at saturating concentrations, suggesting that the two agents may activate shared effector mechanisms. ATA also stimulated proliferation of a PRL-independent Nb2-SP cell line, which is a derivative clone with an elevated constitutive proliferation rate (18). Probably due to this higher proliferation rate, the effect of ATA on Nb2-SP cell growth was modest, averaging 1.6-fold of basal growth rate (p < 0.001, n = 3; data not shown). However, as presented in Fig. 1B, ATA significa ntly counteracted DNA fragmentation in Nb2-SP cells induced by the protein kinase inhibitor staurosporine (Fig. 1B, lanes a–d). PRL was less efficient in preventing staurosporine-induced DNA fragmentation in these cells (Fig. 1B, lane e). From these studies of Nb2 cell biology, we conclude that ATA has marked positive effects on the growth and survival of Nb2 lymphoma cells.

ATA Stimulates Tyrosine Phosphorylation of Jak2—We have previously shown that PRL specifically activates Jak2 in Nb2 cells, whereas several other lymphocyte growth factors activate Jak3 via the common IL2-receptor-γ in these cells (11, 12). We therefore tested the effect of ATA on Jak kinases in Nb2 cells. Quiescent Nb2 cells were treated with or without ATA (250 μM) for 10 min. Individual Jaks were immunoprecipitated from cell lysates, separated by SDS-PAGE, and immunoblotted with antiphosphotyrosine antibodies (Fig. 2A, upper row). Jak2 showed a robust tyrosine phosphorylation response to ATA.
whereas no activation of Jak1, Jak3, or Tyk2 was observed. Of these latter protein kinases, Jak3 is expressed at levels comparable with those of Jak2 as judged from immunoblots (Fig. 2A, lower row) and was markedly activated in Nb2 cells by IL2, IL7, and IL9 (Refs. 11 and 12; data not shown). Jak1 and Tyk2 were also not activated by ATA, but the degree of specificity should be interpreted with caution because Jak1 is expressed only at lower levels and Tyk2 is not readily detectable in Nb2 cells (Fig. 2A, lower row). However, ATA-induced activation of Jak2 in Nb2 cells was rapid. Kinetic analysis showed that peak Jak2 phosphorylation levels were reached within 4 min of stimulation and remained detectable for at least 60 min (Fig. 2B). Additional analyses (not shown) revealed that inducible Jak2 tyrosine phosphorylation could be detected within 1 min of ATA exposure, suggesting that Jak2 is an initial mediator of ATA effects.

**ATA Stimulation of Jak2 Is Associated with Activation of Stat5a and Stat5b**—Having established that ATA stimulates Jak2 in Nb2 cells, we examined the effect of ATA on Stat transcription factors, which are substrates of Jak tyrosine kinases (1, 2). Whereas PRL-induced tyrosine phosphorylation of Stat1, Stat3, and Stat5a/b (Ref. 13; data not shown), treatment of Nb2 cells with ATA induced rapid and robust tyrosine phosphorylation only of Stat5a and Stat5b (Fig. 3, **first**, **third**, **sixth**, and **eighth panels**), reaching maximum levels within 5–15 min of ATA stimulation. Reblotting of the samples with corresponding Stat antibodies verified equal levels of Stat loaded per lane (**second**, **fourth**, **seventh**, and **ninth panels**). Similar to previous observations with PRL-induced Stat5 activation (13), ATA treatment led to a partial mobility retardation of Stat5b but not Stat5a (see **fourth panel**). Furthermore, ATA-induced tyrosine phosphorylation of Stat5a and Stat5b was associated with heterodimerization of the two Stat5 proteins, as demonstrated by inducible coimmunoprecipitation of Stat5a and Stat5b (**fifth panel**). Within 4 min of stimulation, Stat5a was detectable in anti-Stat5b immunoprecipitates. This also corresponds to PRL-induced Stat5a/b heterodimerization in Nb2 cells (13). We conclude that ATA causes tyrosine phosphorylation and heterodimerization of Stat5a and Stat5b but does not mimic the additional Stat1 and Stat3 responses induced by PRL in Nb2 cells.

**ATA Activates MAP Kinases ERK1 and ERK2**—We and others have previously shown that PRL activates Jak2, the SHC-Ras pathway, and MAPK in Nb2 cells (19–21). Treatment of Nb2 lymphocytes with ATA also was associated with a rapid activation of MAP kinase ERK2 and to a lesser extent with activation of ERK1 (Fig. 4A). Protein from lysates of cells that had been treated with ATA (250 μM) for varying times up to 60 min were analyzed by SDS-PAGE and immunoblotting with antibodies to the active forms of ERK1/2 (Fig. 4A, **top panel**). MAPK activation was detectable within 4 min of exposure to ATA and culminated at 15 min. Reblotting of the same samples with an anti-pan ERK antibody verified equal loading and established that ERK2 is the predominantly expressed form of these two ERKs in Nb2 cells (Fig. 4A, **middle panel**). Parallel analysis of the enzymatic activity of MAPK was performed using immunoprecipitation of MAPK followed by an *in vitro* kinase assay with myelin basic protein as a substrate. The **bottom panel** of Fig. 4A shows that MAPK enzyme activity corresponded to that observed with anti-phosphoMAPK antibodies, reaching maximum levels at 15 min, followed by a reduction toward base-line levels at 60 min. Furthermore, we demonstrated that both ATA-induced Stat5a/b and MAPK activation were concentration-dependent, with EC50 values of approximately 100–150 μM for both responses (Fig. 4B). Full biochemical responses by Stat5a/b and ERKs were achieved at 250 μM of ATA, using a 10-min stimulation period. A similar ATA concentration dependence also was observed for Jak2 (data not shown). We conclude that in Nb2 cells, ATA activates MAPK in addition to Jak2-Stat5 in a concentration-dependent manner. A recent study reported that ATA protected against apoptosis and stimulated MAPK in PC12 neurona cells but not in NIH-3T3 fibroblasts (7). It may therefore be of interest to determine whether the Jak2-Stat5 pathway is concurrently activated in PC12 cells.

**ATA Induces Stat5a/b DNA Binding**—To establish whether ATA-induced tyrosine phosphorylation and heterodimerization of Stat5a/b were associated with increased DNA binding, we analyzed the ability of Stat5 proteins to interact with the γ-activated site of the PRL response element of the β-casein gene promoter. Lysates of unstimulated Nb2 cells or cells treated with either ATA (250 μM) or PRL (10 nM) for 10 min were analyzed for binding to a 32P-labeled γ-activated site
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Fig. 4. ATA causes activation of MAP kinases and stimulates phosphorylation of Stat5a/b and ERK1 and ERK2 in a concentration-dependent manner. A, MAPK activation by ATA. Quiescent Nb2 cells were incubated with medium (−) or 250 μM ATA (+) for up to 60 min at 37 °C, and samples from whole cell lysates were separated by SDS-PAGE and blotted with antibodies to phosphorylated MAPK (upper panel). Reblot of stripped samples with anti-pan ERK antibodies is shown in the middle panel. The lower panel shows in vitro MAP kinase assay using [32P] incorporation into myelin basic protein (MBP) as detected by autoradiography. B, concentration-dependent effects of ATA. Quiescent Nb2 cells were incubated with medium (−) or 250 μM ATA (+) at 37 °C for various times as indicated, and lysates were immunoprecipitated with either a Stat5a or a Stat5b serum and blotted with phosphotyrosine antibodies (upper and middle panels). Parallel samples of whole cell lysates were also blotted with α-phosphoMAPK antibodies (lower panel) to detect activated ERK1 and ERK2.

Fig. 5. ATA induces Stat5-DNA binding and stimulates mRNA levels of the Stat5-regulated pim-1 protooncogene. A, EMSA of ATA-inducible protein binding to the PRL response element of the β-casein gene promoter. Quiescent Nb2 cells were incubated with medium (−), 250 μM ATA (+), or 10 nM PRL for 10 min at 37 °C. Lysates corresponding to 10 μg of protein were incubated either with normal rabbit serum (lanes a, b, and h), a Stat1a (lanes c and i), a Stat3 (lanes d and j), a Stat5a (lane e), a Stat5b (lane f), or a Stat5a plus a Stat5b (5a/b, lanes g and k) in combination with an oligonucleotide probe corresponding to the PRL response element of β-casein gene. B, Northern blot analysis of pim-1 mRNA expression. Quiescent Nb2 cells were incubated with 10 nM PRL or 100 μM ATA for varying times up to 4 h at 37 °C. Total mRNA was isolated and analyzed by hybridization with a human pim-1 cDNA probe (upper panel). Both PRL and ATA induced rapid elevation of the 2.8-kb pim-1 mRNA. A larger, transiently induced transcript of unknown identity was also seen at 30 min. Equal loading of mRNA is verified by visualization of copurified 28 S rRNA (lower panel).

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What Is the Mechanism of ATA-induced Jak2-Stat5 Activation?—We have demonstrated that a non-peptide agent, ATA, is capable of activating a single Jak-Stat pathway (Jak2-Stat5) in addition to stimulating the serine kinases ERK1/2 in Nb2 lymphoma cells. Based upon the presently observed temporal relationship between ATA-induced Jak2, Stat5, and MAPK activation, as well as upon current models of Jak-mediated signal transduction (25, 26), activation of Jak2 is probably the most upstream of these events. ATA is a polycarboxylated triphenylmethane derivative with a molecular mass of 473 Da that can polymerize into larger complexes of up to 6,000 Da (27). Because ATA was found not to permeate the intact cell membrane (28), it is presumably activating Jak2 via transmembrane receptors. One possibility is that particular cell surface proteins may be aggregated by ATA to induce the intracellular changes in Jak2 and Stat5 activation. The most likely candidate membrane-spanning proteins are hormone and cytokine receptors of the Jak/STAT pathways upon oligomerization, such as PRL receptors (29).

Is Aggregation of PRL Receptors Key to Mediating the Observed ATA Effect in Nb2 Cells?—ATA does mimic PRL-induced growth of Nb2 cells, and like PRL (9, 30), ATA activates Jak2 and not other Jaks in Nb2 cells. Furthermore, consistent with a shared mechanism of action, when given together at maximally effective concentrations, ATA and PRL do not act synergistically or additively on cell growth, whereas their ef-
fects are additive at submaximal concentrations. On the other hand, ATA exclusively induced Stat5a/b-DNA complexes and, unlike PRL, did not also stimulate tyrosine phosphorylation of Stat1 and Stat3. Furthermore, we have been unable to detect any ATA-induced Jak or Stat activation in human mammary T47D cells (data not shown), which express high levels of PRL receptors and display PRL-induced tyrosine phosphorylation of Stat1, Stat3, and Stat5 (30).2 Preliminary efforts also did not indicate that ATA mimics PRL-induced tyrosine phosphorylation of the PRL receptors in Nb2 cells (data not shown). Finally, additional experiments suggest that PRL receptors may not be a prerequisite, because ATA was able to induce Stat5 phosphorylation in the mouse 32D leukocyte line (data not shown), which does not express PRL receptors (15).

In addition to membrane proteins of the cytokine receptor family, some growth factor receptors with intrinsic tyrosine kinase activity can activate Jak-Stat pathways. For example, EGF activated Jak1 and Stat1 and Stat3 in fibroblasts (31). Very recently, ATA treatment of the SH-SY5Y neuroblastoma cell line was reported to cause tyrosine phosphorylation of ErbB4, a protein of the EGF receptor family (32). Thus, the possibility cannot be excluded that receptors of the EGF family are involved in transmembrane signaling by ATA in Nb2 cells. However, regardless of the mechanism, the observed discriminating activation by ATA of a Jak2-Stat5 pathway suggests an unexpected potential for pharmaceutical targeting of particular Jak-Stat pathways.

In conclusion, this study presents novel evidence for discriminative pharmacological activation of a single Jak2-Stat5 pathway, an effect that was associated with survival and sustained growth of Nb2 lymphocytes. Jak2 activation was associated with a robust activation of Stat5a and Stat5b as measured by tyrosine phosphorylation, heterodimerization, and DNA binding. ATA also induced mRNA levels of the Stat5-regulated pim-1 protooncogene. The present study demonstrates that certain hormone or cytokine effects on Jak-Stat pathways may be additive but not at submaximal concentrations. The results are also consistent with a proposed role for Stat5 in lymphocyte growth and survival (22) and consistent with the fact that Jak2 is a direct upstream activator of Stat5a and Stat5b. Future studies are directed at validating biological applications of ATA and will explore the molecular mechanism of ATA-induced Jak2-Stat5 activation.

REFERENCES
1. Ihle, J. N. (1995) Nature 377, 591–594
2. Larner, A. C., and Finbloom, D. S. (1995) Biochem. Biophys. Acta 1266, 278–287
3. March, P., Villa, A., Gilliani, S., Sacco, M. G., Frattini, A., Porta, F., Ugazio, A. G., Johnston, J. A., Candotti, F., O'Shea, J. J., et al. (1995) Nature 377, 55–68
4. Meran, M. A., White, J. M., Sheehan, K. C., Bach, E. A., Rodig, S. J., Dighe, A. S., Kaplan, D. H., Riley, J. K., Greenland, A. C., Campbell, D., Carver-Moore, K., Du Bois, R. N., Clark, R., Aguet, M., and Schreiber, R. D. (1996) Cell 84, 431–442
5. Liu, X., Robinson, G. W., Wagner, K. U., Barrett, L., Wynshaw-Boris, A., and Hennighausen, L. (1997) Genes Dev. 11, 179–186
6. Meydan, N., Grunberger, T., Dadi, H., Shabah, M., Arpaia, E., Lapidot, Z., Leeder, J. S., Freestman, M., Cohen, A., Gazit, A., Levitizki, A., and Roifman, C. M. (1996) Nature 379, 645–648
7. Okada, N., and Koizumi, S. (1995) J. Biol. Chem. 270, 16464–16469
8. Lavoie, H. A., and Witorsch R. J. (1995) Proc. Soc. Exp. Biol. Med. 209, 257–269
9. Rui, H., Kirken, R. A., and Farrar, W. L. (1994) J. Biol. Chem. 269, 5364–5368
10. Lebrun, J. A., Ali, S., Sofer, L., UILrich, A., and Kelly, P. A. (1994) J. Biol. Chem. 269, 14921–14926
11. Kirken, R. A., Rui, H., Malabarba, M. G., and Farrar, W. L. (1994) J. Biol. Chem. 269, 19136–19141
12. Kirken, R. A., Rui, H., Malabarba, M. G., Howard, O. M., Kawamura, M., O'Shea, J. J., and Farrar, W. L. (1995) Cytokine 7, 689–700
13. Kirken, R. A., Malabarba, M. G., Xu, J., Liu, X., Farrar, W. L., Hennighausen, L., Larner, A. C., Grimley, P. M., and Rui, H. (1997) J. Biol. Chem. 272, 14098–15003
14. Gout, P. W., Beer, C. T., and Noble, R. L. (1980) Cancer Res. 40, 2433–2436
15. Da Silva, L., Howard, O. M., Rui, H., Kirken, R. A., and Farrar, W. L. (1994) J. Biol. Chem. 269, 18267–18270
16. Miller, S. A., Dykes, D. D., and Polesky, H. I. (1988) Nucleic Acids Res. 16, 1216–1217
17. Rui, H., Djeu, J. Y., Evans, G. A., Kelly, P. A., and Farrar, W. L. (1992) J. Biol. Chem. 267, 24076–24081
18. Walker, A., Croze, F., and Friesen, H. G. (1987) Endocrinology 120, 2359–2397
19. Erwin, R. A., Kirken, R. A., Malabarba, M. G., Farrar, W. L., and Rui, H. (1995) Endocrinology 136, 3512–3518
20. Buckley, A. R., Rao, Y. P., Buckley D. J., and Gout, P. W. (1994) Biochem. Biophys. Res. Commun. 204, 1158–1164
21. Carey, G. B., and Liberti, J. P. (1995) Biochem. Biophys. Biophys. 316, 179–189
22. Muli A. L., Wakao, H., Kinoshita, T., Kitamura, T., and Miyajima, A. (1996) EMBO J. 15, 2425–2433
23. Buckley, A. R., Buckley, D. J., Leff, M. A., Hoover, D. S., and Magnuson, N. S. (1995) Endocrinology 136, 5252–5259
24. Wingett, D., Reeves, R., and Magnuson, N. S. (1992) Nucleic Acids Res. 20, 3183–3189
25. Gouilleux, F., Wakao, H., Mundt, M., and Groner, B. (1994) EMBO J. 13, 4361–4369
26. Winston, L. A., and Hunter, T. (1995) J. Biol. Chem. 270, 30387–30400
27. Gun, Z., Weinheimer, M. J., Phillips, M. D., and Kroll, M. H. (1993) Thromb. Res. 71, 77–88
28. Apirion, D., and Dohner, D. (1975) in Antibiotics (Corcoran, J. W., and Hahn, H. E., eds) Vol. III, pp. 327–340, Springer-Verlag, New York
29. Rui, H., Lebrun, J. A., Kirken, R. A., Kelly, P. A., and Farrar, W. L. (1994) Endocrinology 135, 1299–1306
30. Da Silva, L., Rui, H., Erwin, R. A., Howard, O. M., Kirken, R. A., Malabarba, M. G., Hackett, R. H., Larner, A. C., and Farrar, W. L. (1996) Mol. Cell Endocrinol. 117, 131–140
31. Leisman, D. W., Pisharody, S., Flickinger, T. W., Commanne, M. A., Schlessinger, J., Kerr, I. M., Levy, D. E., and Stark, G. R. (1996) Mol. Cell Biol. 16, 369–375
32. Okada, N., and Koizumi, S. (1997) Biochem. Biophys. Res. Commun. 230, 266–269