Evidence for a Signaling Role for the α Chains of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Interleukin-3 (IL-3), and IL-5 Receptors: Divergent Signaling Pathways Between GM-CSF/IL-3 and IL-5


In the present study, we have used a human erythroleukemia cell line, TF-1, that proliferates in response to granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-3 (IL-3), and interleukin-5 (IL-5) to investigate the role of receptors for these cytokines in signal transduction mechanisms involved in proliferative responses. The receptors for GM-CSF, IL-3, and IL-5 each possess a cytokine specific α subunit, but all three share a common β chain. Using an immunoblotting system designed to detect phosphotyrosine containing proteins and a permeabilized cell system to detect rapid changes in phosphate turnover on proteins, we show that while GM-CSF and IL-3 use tyrosine phosphorylation to mediate mitogenic signal transduction, IL-5 uses tyrosine dephosphorylation in its signaling pathway. The use of different signaling pathways by these cytokines can be confirmed in a biologic system whereby the proliferation induced in culture by GM-CSF and IL-3 is inhibited by tyrosine kinase inhibitors, but that induced by IL-5 is enhanced. Conversely, GM-CSF- and IL-3-induced proliferation is stimulated by a tyrosine phosphatase inhibitor, yet IL-5-induced proliferation is inhibited. Inhibitors of protein kinase C inhibit IL-3- and GM-CSF-, but not IL-5-induced proliferation. We suggest that, because all these cytokines share the identical β chain of their receptors, the cytokine specific α chain mediates the linkage of each receptor to the individual biochemical signal transduction pathways responsible for the different biologic activities of these cytokines.

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MATERIALS AND METHODS

Cell line. The human erythroleukemia cell line TF-1 was generously supplied by Dr T. Kitamura (University of Tokyo, Tokyo, Japan). This cell line is growth factor-dependent and responds to IL-3, IL-5, and GM-CSF, as well as several other cytokines.

Maintenance of TF-1 cells. TF-1 cells can be maintained in RPMI 1640 medium (GIBCO, Paisley, UK), supplemented with 5% fetal calf serum (FCS) and penicillin/streptomycin at 37°C, 5% CO₂ humidified atmosphere. In addition, the cells require the presence of a cytokine for survival and proliferation. Depending on the experiment, cells were grown in either 2 ng/mL GM-CSF, 100 ng/mL leukemia inhibitory factor (LIF), or 5 ng/mL IL-5.

Cytokines. GM-CSF, IL-3, IL-5, and LIF used in this study were kind gifts from Schering Plough Inc (Kenilworth, NJ), Immunex Corp (Seattle, WA), Sandoz Pharma Ltd (Basel, Switzerland), and Glaxo (Greenford, UK). All were purified to apparent homogeneity and rDNA derived.

Cytokine starvation of cells. Before use in experiments, TF-1...
cells were washed twice in RPMI 1640 medium supplemented with 5% FCS. The cells were then resuspended at $1 \times 10^5$ cells/mL in RPMI 1640 medium/5% FCS and incubated overnight at 37°C, 5% CO$_2$ humidified atmosphere.

Detection of protein dephosphorylation using permeabilized cells. Cytokine-starved cells were washed once with serum-free RPMI medium, aliquoted in $1 \times 10^5$ cell lots and centrifuged in 2-mL Eppendorf tubes at 500g for 5 minutes at room temperature. Each cell pellet was resuspended in 8 μL of a buffered reaction mixture containing calcium, magnesium, and manganese ions as described elsewhere. A total of 2 μL of L-α-lysophosphatidylcholine (Lipid Products Ltd, Redhill, Surrey, UK), 1 mg/mL in water was added and the suspension gently mixed. The reaction was initiated by the addition of 5 μCi (γ-32P) adenosine triphosphate (ATP). Labeling occurred for 5 minutes at room temperature before the addition of cytokines with 0.1% ovalbumin used as a diluent and control throughout. The reaction was stopped by the addition of 20 μL of an ice-cold solution containing 2% Nonidet P40, 20 mmol/L sodium orthovanadate, 1 mmol/L phenylmethanesulfonyl fluoride, 5 mmol/L p-nitrophenyl phosphate, 20 mmol/L EDTA, and 50 μmol/L ATP. The detergent-treated cells were centrifuged at 1,500g (4°C) for 5 minutes to separate the detergent insoluble material (nuclei and cytoskeleton) from the detergent soluble material (cytosol plus solubilized membrane and organelles). The detergent soluble fractions were analyzed by electrophoresis in 7.5% polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS) under reducing conditions. The resulting gels were fixed, stained with Page Blue G-90 (Pharmacia, St Albans, UK), destained, and dried under heat and vacuum. The dried gels were the autoradiographed using Kodak X-Omat S film (Eastman Kodak, Rochester, NY) at -70°C using intensifying screens. Each experiment was repeated at least three times.

Immunoblot detection of phosphotyrosine containing proteins. Cytokine-starved TF-1 cells were washed twice in RPMI 1640 medium and two times in cold phosphate-buffered saline (PBS) for each assay condition. A total of 0.5 to $1 \times 10^5$ cells were resuspended in 400 μL of PBS. Cytokines or PBS controls were added and the cells incubated for 10 minutes on ice. Cells were then pelleted and resuspended in 150 μL of lysis buffer containing 20 mmol/L Tris/HCl pH 8.0, 137 mmol/L NaCl, 1% NP40, 1 mmol/L phenylmethanesulfonyl fluoride (PMSF), 10 mmol/L EDTA, 100 mmol/L sodium fluoride, and 2 mmol/L sodium orthovanadate. Samples were separated by SDS-polyacrylamide gel electrophoresis (PAGE), transferred onto nitrocellulose filters, and incubated with a specific phosphotyrosine monoclonal antibody (UBI, Lake Placid).

Fig 2. Time courses and dose responsiveness of cytokine-induced tyrosine phosphorylation events in TF-1 cells growing in GM-CSF or LIF. TF-1 cells were cultured in either 2 ng/mL GM-CSF (A, B, or C) or 5 ng/mL LIF (D). Cells were washed, starved overnight, and exposed to various cytokines for the times indicated. Lysates were then prepared and phosphotyrosine containing proteins detected using anti-phosphotyrosine antibody and immunoblotting as described in Materials and Methods. (A) Lane 1, no additions, cells incubated for 1 hour; lane 2, 50 ng/mL IL-5 added to cells for 1 hour; lane 3, 5 ng/mL IL-5 added to cells for 1 hour; lane 4, 50 ng/mL GM-CSF added to cells for 1 hour; lane 5, 5 ng/mL GM-CSF added to cells for 3 hours. (B) IL-3-induced tyrosine phosphorylation events. Lane 1, no addition, cells incubated for 10 minutes; lane 2, 50 ng/mL IL-3, 10 minutes; lane 3, 25 ng/mL IL-3, 10 minutes; lane 4, 5 ng/mL IL-3 for 10 minutes; lane 5, no addition, cells incubated 30 minutes; lane 6, 50 ng/mL IL-3, 30 minutes; lane 7, 25 ng/mL IL-3, 30 minutes; lane 8, 5 ng/mL IL-3, 30 minutes; lane 9, no addition, cells incubated for 1 hour; lane 10, 50 ng/mL IL-3, 1 hour; lane 11, 25 ng/mL IL-3, 1 hour; lane 12, 5 ng/mL GM-CSF 1 hour. (C) Cytokine-induced tyrosine phosphorylation events induced in TF-1 cells maintained in LIF. Lane 1, no addition, cells incubated for 10 minutes; lane 2, 10 ng/mL GM-CSF, 10 minutes; lane 3, IL-5 25 ng/mL 10 minutes; lane 4, IL-3 10 ng/mL 10 minutes; lane 5, 50 ng/mL GM-CSF, 30 minutes; lane 6, 50 ng/mL IL-5, 30 minutes.
Phosphotyrosine containing proteins were detected using an enhanced chemoluminescence (ECL) detection kit (Amersham International, Amersham, UK). Each experiment was repeated at least three times.

Assay of cellular proliferation. TF-1 cells were washed twice with RPMI 1640 medium and resuspended at a final concentration of $1 \times 10^6$ cells/mL in RPMI 1640 medium containing 5% FCS and antibiotics. Titrations of cytokines were made in 100-µL volumes in a microtiter plate, making serial dilutions from 1,000 U/mL down to 1 U/mL. Inhibitors, genisteine, chelerythrine, staurosporin, herbimycin A, and sodium orthovanadate (Sigma, Poole, UK) were added to cells for 1 hour before addition of 100 µL cell suspension to the microtiter plates (unless indicated otherwise) and the plates incubated for 48 hours at 37°C in a humidified CO$_2$ atmosphere. Tritiated thymidine (0.5 µCi) was added to each
well, and the plates were returned to the incubator for approximately 4 hours. The contents of each well were harvested onto filter mats, and the radioactivity incorporated into DNA was determined by liquid scintillation counting. Each experiment illustrated was repeated at least three times. The 95% confidence limits for bioassay variation were 85% to 120% of each symbol illustrated. For clarity, error bars have not been included in the figures, but no error bar fell outside the 95% confidence limits, except at a few plateau level points where maximal variation tends to occur.

Quantification of p80 dephosphorylation. Autoradiograms were scanned using an LKB (Pharmacia) UltraScan XL enhanced laser densitometer. Quantitative integration was performed using gel scan 2.1 software (Pharmacia), taking into account lane-to-lane variation. For comparative purposes, the density of p80 in cells undergoing the treatments described was compared with p80 in control cells and expressed as a percentage of the density of the control band.

RESULTS

Biologic response of TF-1 cells grown in different cytokines. TF-1 cells proliferate in response to different cytokines to different degrees, with GM-CSF being the most potent stimulator of proliferation (Fig 1A). GM-CSF, IL-3, and IL-5 are all able to induce proliferation of TF-1 cells and can be used to maintain the cells in culture for extended periods of time. There is essentially little difference in the response of TF-1 cells to either GM-CSF, IL-3, or IL-5 if the cells are maintained in culture with either GM-CSF, IL-5, or LIF, except that the IL-5 response is slightly increased in cells grown in IL-5 or LIF (Fig 1B and 1C).

Tyrosine phosphorylation events induced by GM-CSF and IL-3, but not IL-5. Addition of GM-CSF to TF-1 cells maintained in GM-CSF primarily induces the phosphorylation of tyrosine residues on proteins of molecular weight 158 and 162 kD (Fig 2A, lanes 4 and 5). Stimulation of tyrosine phosphorylation at 1 hour occurs with 50 ng/mL GM-CSF, and phosphorylation is detectable at 500 pg/mL (Fig 2A, lane 6). Although a dose response clearly occurs with GM-CSF, it is difficult to correlate with biologic activity because the individual experiments (phosphorylation and bioassay) require very different concentrations of cells (10^6 cells/point for phosphorylation, 0.2 x 10^{9} cells for bioassay) so that the cell number/cytokine concentration balance is artificially high in phosphorylation experiments. The response to GM-CSF is also time dependent, with higher concentrations of GM-CSF able to induce maximal phosphorylation within 10 minutes, but at 5 ng/mL, GM-CSF maximal stimulation occurs at 60 minutes (Fig 2C, lane 12).

IL-3 induces similar tyrosine phosphorylation events to GM-CSF in cells grown in GM-CSF with the tyrosine phosphorylation of 158 and 162 kD proteins within 10 minutes of addition of IL-3 to the cells (Fig 2B, lanes 2 through 4). It is interesting to note that the dose response of the tyrosine phosphorylation in TF-1 cells to IL-3 is less sensitive than that of GM-CSF (Fig 2B and C). This correlates with their ability to induce proliferation of TF-1 cells in cell culture (Fig 1A). Unlike GM-CSF or IL-3, IL-5 does not induce tyrosine phosphorylation at any of the doses tried, even at 50 ng/mL (Fig 2A, lane 2). The lack of response is not due to time dependency because no phosphorylation occurs even after incubation for 3 hours at 50 ng/mL IL-5 (Fig 2A, lane 10). The lack of induction of tyrosine phosphorylation by IL-5 is also not due to maintenance of cells in GM-CSF. Cells grown in LIF do not respond with tyrosine phosphorylation on addition of IL-5 (Fig 2D, lanes 3 and 6), but on addition of IL-3 and GM-CSF, LIF-maintained cells produce similar phosphorylation events as cells grown in GM-CSF (Fig 2D, lanes 2, 4, and 5).

Tyrosine phosphorylation events induced by IL-5, but not GM-CSF or IL-3. Using a well-characterized permeabilized cell system designed to detect rapid changes in phosphate turnover, it can be shown that addition of IL-5 to prerdialabeled permeabilized TF-1 cells grown in either GM-CSF, LIF, or IL-5 results in the rapid removal of phosphate from an 80-kD protein (p80) (Fig 3A, lane 1; Fig 3B, lane 2; Fig 3C, lanes 9 and 10). p80 has been extensively characterized elsewhere and the IL-5–induced dephosphorylation event is almost identical in character to that described for IL-4. Removal of radiolabel from phosphotyrosine residues was confirmed by the total alkali resistance of the radiolabel on p80 before total removal induced by IL-5, as detected by quantitative densitometric scanning of the autoradiograms (data not shown). This response is extremely rapid, occurring within 30 seconds (Fig 4) and closely follows the dose response of the biologic activity of IL-5 on the TF-1 cells grown under the different conditions as illustrated (Figs 1 and 4). However, neither IL-3 nor GM-CSF has any influence on the phosphate turnover of p80 even at very high doses of cytokine (Fig 3A, lanes 3 and 6; Fig 3B, lanes 3 and 6; Fig 3C, lanes 4 and 6). Time does not appear to be a factor in the nonresponsiveness of p80 to IL-3 and GM-CSF because if cells are left in their presence for up to 1 hour, no change in the phosphorylation status of p80 can be detected (Fig 3A, lane 7; Fig 3B, lane 4; Fig 3C, lane 2 and Fig 4C). Again, there is no difference in response between TF-1 cells grown in GM-CSF, LIF, or IL-5 (Figs 3 and 4).

Effects of tyrosine kinase inhibitors on cytokine induced proliferation of TF-1 cells in culture. Starved TF-1 cells preexposed to the tyrosine kinase inhibitor genistein exhibit an enhanced proliferation compared with unexposed cells when subsequently grown in the presence of IL-5 (Fig 5A). Enhanced proliferation increases in a dose-responsive manner up to 50 ng/mL genistein, after which toxicity reduces the viability of the TF-1 cells. However, if cells exposed to genistein were subsequently cultured in the presence of either GM-CSF or IL-3, their proliferation is dramatically decreased in comparison to unexposed cells (Fig 5A). This suggests that the inhibition of tyrosine kinases before the addition of cytokines is able to inhibit the proliferative signal transduction induced by GM-CSF and IL-3, but enhance that of IL-5. This phenomenon is time specific, as genistein-induced effects decrease the shorter the incubation time with genistein before cytokine addition. If genistein is added with or after cytokine, no effect occurs (Fig 5B). This suggests that genistein must cross the cell membrane and inhibit the tyrosine kinases before the cytokine signaling pathways are activated. Once the kinase pathways have been activated, their subsequent inhibition no longer inhibits cellular prolif-
Fig 3. Cytokine-induced dephosphorylation of an 80-kD protein in TF-1 cells maintained in GM-CSF, LIF, or IL-5. TF-1 cells were maintained in the different cytokines indicated as described in Materials and Methods. Cells were washed extensively and incubated overnight in the absence of cytokines before the permeabilization procedure as described in Materials and Methods. Permeabilized cells were incubated in the presence or absence of different cytokines with (γ-32P) ATP for various times. Cells were then lysed, proteins separated on SDS-PAGE, and labeled proteins detected by autoradiography as described in Materials and Methods. (A) TF-1 cells maintained in GM-CSF. Lane 1, 0.1% ovalbumin, cells incubated in the presence of (γ-32P) ATP for 10 minutes; lane 2, 5 ng/mL IL-3, 10 minutes; lane 3, 100 ng/mL IL-3, 10 minutes; lane 4, 50 ng/mL IL-3, 10 minutes; lane 5, 100 ng/mL GM-CSF, 10 minutes; lane 6, 50 ng/mL GM-CSF, 1 hour; lane 7, 10 ng/mL GM-CSF, 10 minutes. (B) TF-1 cells maintained in LIF. Lane 1, 0.1% ovalbumin, cells incubated in the presence of (γ-32P) ATP for 10 minutes; lane 2, 5 ng/mL IL-5, 10 minutes; lane 3, 50 ng/mL GM-CSF, 10 minutes; lane 4, 100 ng/mL GM-CSF, 1 hour; lane 5, 10 ng/mL GM-CSF, 10 minutes. (C) TF-1 cells maintained in IL-5. Lane 1, 0.1% ovalbumin, cells incubated in the presence of (γ-32P) ATP for 10 minutes; lane 2, 5 ng/mL GM-CSF, 1 hour; lane 3, 50 ng/mL GM-CSF, 10 minutes; lane 4, 100 ng/mL GM-CSF, 10 minutes; lane 5, 10 ng/mL GM-CSF, 10 minutes; lane 6, 100 ng/mL IL-3, 10 minutes; lane 7, 50 ng/mL IL-3, 10 minutes; lane 8, 10 ng/mL IL-3, 10 minutes; lane 9, 1 ng/mL IL-5, 10 minutes; lane 10, 10 ng/mL IL-5, 10 minutes.
seration. Attempts to use another tyrosine kinase inhibitor, herbimycin A, were hampered by the very restricted dose response between having no effect and toxicity. No effect occurred below 10 ng/mL, but severe toxicity occurred at 50 ng/mL. Although there were no effects on IL-5--induced proliferation at 10 to 25 ng/mL herbimycin A, GM-CSF and IL-3--induced proliferation was inhibited between 10% to 20%. However, even slight shifts in toxicity from assay to assay meant that this response was not statistically significant.

Effects of a tyrosine phosphatase inhibitor on cytokine induced proliferation of TF-1 cells in culture. Preincubation for 1 hour of starved TF-1 cells with varying concentrations of sodium orthovanadate resulted in divergent effects on subsequent proliferation induced by IL-3/GM-CSF or IL-5. The proliferation induced by IL-5 was inhibited by preincubation with orthovanadate (Fig 6), while that of IL-3 and GM-CSF was either not affected at low doses or there was significant stimulation (P < .01) at doses between 10 to 100 nmol/L. Toxic effects occurred for all cells subsequently

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**Fig 4.** Dose response and time course of p80 dephosphorylation in TF-1 cells maintained with either GM-CSF or LIF. Tyrosine dephosphorylation assays were performed as described in Materials and Methods and illustrated in Fig 3. (A) Cells maintained in GM-CSF before assay. Densitometric scans were performed on the 80-kD region of autoradiograms produced from cells exposed to varying concentrations of GM-CSF (□), IL-3 (○), or IL-5 (▲). The density of the p80 band in treated cells was compared with control cells without cytokine addition. This, together with differences in lane-to-lane density, was calculated quantitatively using gel scan 2.1 software (LKB) on an ultrascan XL enhanced laser densitometer (LKB). (B) Cells maintained in LIF before assay. Densitometric scans were performed on the 80-kD region of autoradiograms produced from cells exposed to varying concentrations of GM-CSF (□), IL-3 (○), or IL-5 (▲). (C) Cells were incubated with cytokines for various times before lysing and further processing as described. Densitometric scans were performed on the 80-kD region on autoradiograms from cells grown in GM-CSF exposed to 100 ng/mL GM-CSF (□), 100 ng/mL IL-3 (○), or 1 ng/mL IL-5 (▲) or cells grown in LIF exposed to 100 ng/mL GM-CSF (□) or 10 ng/mL IL-5 (▲).

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**Fig 5.** Dose response and time course of effects of genistein on TF-1 cells in culture. (A) TF-1 cells maintained in GM-CSF were washed and starved of cytokine overnight as described. The cells were incubated at 37°C for 1 hour with varying concentrations of genistein before the addition of either 200 pg/mL GM-CSF (□), 2 ng/mL IL-3 (○), or 2 ng/mL IL-5 (▲). (B) TF-1 cells maintained in GM-CSF were washed and starved of cytokine overnight as described. The cells were either preincubated at 37°C for various times with 50 ng/mL genistein before addition of cytokine or 50 ng/mL genistein was added at the times indicated after cytokine was added to cells. Cytokines were 200 pg/mL GM-CSF (□), 2 ng/mL IL-3 (○), or 2 ng/mL IL-5 (▲). The cells were then incubated for a further 48 hours and proliferation measured by "H-thymidine incorporation as described. Proliferation is expressed as the percentage cytokine-induced proliferation of genistein--exposed cells compared with proliferation each cytokine induced in cells that had not been exposed to genistein before cytokine addition.
Effects of PKC inhibitors on cytokine induced proliferation of TF-1 cells in culture. Preincubation for 1 hour of starved TF-1 cells with staurosporin did not affect the subsequent induction of proliferation by IL-5, but significantly inhibited that induced by either IL-3 or GM-CSF (Fig 7A). This occurred in a dose-responsive manner, with the greatest effects occurring at 15 nmol/L. Staurosporin appeared toxic at doses greater than 50 nmol/L for all three cytokines (Fig 7A). As for the other inhibitors, the shorter the preincubation time to the time of addition of cytokine, the smaller the effects. It is interesting to note that while no effects occurred with IL-5 stimulation at any time point, a small, but significant \( P < .1 \) increase in proliferation with GM-CSF and IL-3 occurs if 10 to 15 nmol/L staurosporin is added at the same time as the cytokine (Fig 7B). This may be caused by the inhibition of the negative feedback role of PKC after the positive PKC signal has been sent. The use of another PKC inhibitor, chelerythrine, has even more exaggerated problems associated with it than herbimycin A. In TF-1 cells, 1.5 \( \mu \)mol/L chelerythrine is completely toxic, while at 0.5 \( \mu \)mol/L there are no effects. While we consistently see inhibition of GM-CSF- and IL-3-induced proliferation between these doses, there are no effects for IL-5. However, these responses cannot be regarded as significant if one seriously takes into account the very small effective dose range and the large toxic effects of the compound.

**DISCUSSION**

The data described here clearly illustrate a dichotomy between the signaling pathways of IL-3/GM-CSF and IL-5. It has been shown by several studies that IL-3 and GM-CSF use tyrosine phosphorylation as part of their mitogenic signal transduction in a wide range of cell types.\(^{10,20,25}\) We show that with TF-1 erythroleukemia cells, GM-CSF and IL-3 induce the phosphorylation of several proteins on tyrosine residues. The mitogenic signal transduction pathway of IL-5 in human cells has not been reported to date, although data on signaling in murine cells have been reported.\(^{30,31}\) We show that IL-5 induces the rapid tyrosine dephosphorylation of an 80-kD protein previously described as being involved in the signal transduction pathway for IL-4. The data available for IL-5 signal transduction are similar to those for IL-4, namely that in the murine system both IL-5 and IL-4 induce tyrosine phosphorylation events, and yet no such events have been reported for human cells using human cytokines.\(^{20}\) This illustrates the difficulty in applying data provided by murine systems to events that occur in human cells. This is particularly true for the IL-5, IL-3, and GM-CSF receptor system for which the murine system is apparently more complicated, as it includes an IL-3-specific \( \beta \) chain that seems not to exist in humans, in addition to a \( \beta \) chain specific for all three cytokines.\(^{3,32}\) Transfection of NIH3T3 murine cells with human \( \alpha \) and \( \beta \) GM-CSF receptor chains results in some signal transduction events on addition of human GM-CSF, but human GM-CSF does not induce the cells to proliferate.\(^{33}\) This report suggests that the human receptors can link to signaling pathways in murine cells, but either cannot activate all the pathways required for proliferation or that human receptors link to different pathways that are unable to induce proliferation in murine cells. Transfection of Xenopus oocytes with the human \( \alpha \) chain of the GM-CSF receptor leads to GM-CSF-induced responses on glucose transport that are phosphorylation independent.

This report therefore illustrates a signaling role for the \( \alpha \) chain alone. However, it is difficult to correlate with proliferative signal transduction events in this case, as the oocytes do not proliferate in response to GM-CSF.\(^{17}\) How differences between such receptor systems influence mitogenic signal transduction (particularly for IL-3) has yet to be established. Therefore, the differences observed between the signaling pathways for GM-CSF/IL-3 and IL-5 may be specific for human cells. The differences between the biochemical events involved in the cytokine signaling were confirmed in culture whereby tyrosine kinase inhibitors inhibited IL-3 and GM-CSF-induced proliferation but enhanced that of IL-5 and visa versa for the tyrosine phosphatase inhibitors, sodium orthovanadate. It has been shown that addition of vanadate...
can induce the transient proliferation of IL-3–dependent cells, thus consolidating our findings.34

It is also apparent that GM-CSF and IL-3 use PKC as part of their signal transduction pathways, whereas IL-5 does not. IL-3 has been shown elsewhere to activate PKC in human MO7e megakaryoblastic cells15,26 and GM-CSF activates PKC in human α chain transfected Ba/F3 cells.26 It is interesting to note the differences in the effects of PKC inhibitors depending on what time they are added to the cells in relation to addition of the stimulatory cytokine. If cells are preincubated with the inhibitors, the cytokine-induced proliferation (by GM-CSF or IL-3) is inhibited, suggesting that PKC plays a positive signaling role. However, if the inhibitors are added simultaneously with the cytokine, the resulting proliferation is slightly enhanced. It has been shown that PKC can phosphorylate itself,37 but the role of PKC autophosphorylation is at present controversial and has been shown not to be a prerequisite for activation. It may, however, be involved in downregulation or degradation of PKC.38 If so, the prevention of autophosphorylation by inhibitors may extend the positive actions of activated PKC in IL-3 and GM-CSF signal transduction and enhance the proliferation induced by these cytokines. PKC activation before cytokine addition has been shown to inhibit GM-CSF–induced tyrosine phosphorylation events illustrating that PKC can activate an inhibitory pathway.39 This report further consolidates our findings by illustrating that sodium orthovanadate enhances proliferation induced by either GM-CSF or IL-3.40

The difference in signaling pathways between IL-3/GM-CSF and IL-5 is unlikely to be caused by differences in receptor numbers even though there are quantitative differences in actual receptor numbers for these cytokines on TF-1 cells. Receptor numbers for starved TF-1 cells maintained in GM-CSF used throughout this study are 1,552 ± 343 high-affinity (kd 58 ± 23 pmol/L), 6,358 ± 2,101 low-affinity GM-CSF receptors (kd 1.52 ± 3 nmol/L; n = 4); 850 high-affinity IL-3 receptors (kd 496 pmol/L; n = 2), and 446 ± 47 high-affinity IL-5 receptors (kd 227 ± 37 pmol/L; n = 7) (D. Linch and A. Khwaja, personal communication, February 1995). These receptor levels are close to those described elsewhere for TF-1 cells.41,42 Although IL-5 and IL-3 have different numbers of receptors and affinities, they show little difference in their ability to induce proliferation of TF-1 cells, suggesting that receptor numbers are not too important in mediating quantitative differences in signal transduction. More important is the fact that the signaling pathways described here between IL-3/GM-CSF and IL-5 are entirely different (not simply a matter of dose response) so that regardless of the number of receptors, we suggest that IL-3/GM-CSF receptors are linked to one signaling pathway and IL-5 to another.

Therefore, although IL-3, GM-CSF, and IL-5 share a common receptor β chain, GM-CSF and IL-3 seem to share one signaling pathway while IL-5 uses another apparently distinct pathway. Binding of the cytokine to either chain of the receptor in isolation is not enough to transduce mitogenic signals.16,24,43 It is thought that binding of a cytokine to its α receptor subunit leads to a strong association between α

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**Fig 7.** Dose response and time response of effects of staurosporin on TF-1 cells in culture. (A) TF-1 cells maintained in GM-CSF were washed and starved as described. The cells were incubated at 37°C for 1 hour in the presence of varying concentrations of staurosporin before the addition of either 200 pg/mL GM-CSF (●), 2 ng/mL IL-3 (●), or 2 ng/mL IL-5 (●). The cells were incubated for a further 48 hours and proliferation measured by [3H]-thymidine incorporation as described. Proliferation is expressed as the percentage cytokine-induced proliferation of staurosporin-exposed cells compared with the proliferation induced by each cytokine in cells that had not been exposed to staurosporin before cytokine addition. (B) TF-1 cells maintained in GM-CSF were washed and starved of cytokine overnight as described. Various concentrations of staurosporin were added to cells at the same time as either 200 pg/mL GM-CSF (●), 2 ng/mL IL-3 (●), or 2 ng/mL IL-5 (●). The cells were incubated for a further 48 hours and proliferation measured by [3H]-thymidine incorporation as described. Proliferation is expressed as the percentage cytokine-induced proliferation of staurosporin-exposed cells compared with the proliferation induced by each cytokine in cells that had not been exposed to staurosporin before cytokine addition.
and β subunits that leads to signal transduction.32,44 Both the cytoplasmic domains of the α and β chains are required for signal transduction, suggesting that the α subunits may influence the transduction of cytokine specific signals.32,43 The data here suggest that the cytokine specific α chain does, indeed, mediate the linkage of the cytokine receptors to different signaling pathways. Whether this occurs by direct binding of the α chain to signaling enzymes or by the α chain modifying the activity of the β chain is currently under investigation.

Common receptor subunits are not unique to the GM-CSF/IL-3/IL-5 receptors. IL-6, LIF, Oncostatin M, ciliary neurotropic factor, and IL-11 all share a common receptor chain, gp130.45-47 IL-2, -4, -13, and -15 all appear to share a common γ chain that may be involved in signal transduction.48-49 It remains to be seen if, like GM-CSF, IL-3, and IL-5, individual cytokines have overlapping signaling mechanisms or use different pathways and whether the specific cytokine receptor chains within these systems mediate these differences.

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REFERENCES

7. Tavernier J, Devos R, Cornelis S, Van der Heyden J, Fiers W, Plaatnick G: A human high affinity Interleukin-5 receptor (IL-5R) is composed of an IL-5-specific α chain and a β chain shared with the receptor for GM-CSF. Cell 66:1175, 1991
29. Linnekin D, Farrar WL: Signal transduction of human interleukin-3 and granulocyte-macrophage colony-stimulating factor...
33. Eder M, Griffin JD, Ernst TJ: The human granulocyte-macrophage colony-stimulating factor receptor is capable of initiating signal transduction in NIH3T3 cells. EMBO J 12:1647, 1993
44. Sat0 N, Sakamaki K, Terada N, Arai K, Miyajima A: Signal transduction by the high-affinity GM-CSF receptor: Two distinct cytoplasmic regions of the common β subunit responsible for different signaling. EMBO J 12:4181, 1993
49. Zurawski G, de Vries JE: Interleukin-13, an interleukin-4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today 15:19, 1994
Evidence for a signaling role for the alpha chains of granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and IL-5 receptors: divergent signaling pathways between GM-CSF/IL-3 and IL-5

A Mire-Sluis, LA Page, M Wadhwa and R Thorpe