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Hematopoietic Cell Phosphatase Associates with the Interleukin-3 (IL-3) Receptor β Chain and Down-Regulates IL-3-Induced Tyrosine Phosphorylation and Mitogenesis

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Hematopoietic cell phosphatase (HCP) is a tyrosine phosphatase with two Src homology 2 (SH2) domains that is predominately expressed in hematopoietic cells, including cells whose growth is regulated by interleukin-3 (IL-3). The potential effects of HCP on IL-3-induced protein tyrosine phosphorylation and growth regulation were examined to assess the role of HCP in hematopoiesis. Our studies demonstrate that, following ligand binding, HCP specifically associates with the β chain of the IL-3 receptor through the amino-terminal SH2 domain of HCP, both in vivo and in vitro, and can dephosphorylate the receptor chain in vitro. The effects of increasing or decreasing HCP levels in IL-3-dependent cells were assessed with dexamethasone-inducible constructs containing an HCP cDNA in sense and antisense orientations. Increased HCP levels were found to reduce the levels of IL-3-induced tyrosine phosphorylation of the receptor and to dramatically suppress cell growth. Conversely, decreasing the levels of HCP increased IL-3-induced tyrosine phosphorylation of the receptor and marginally increased growth rate. These results support a role for HCP in the regulation of hematopoietic cell growth and begin to provide a mechanistic explanation for the dramatic effects that the genetic loss of HCP, which occurs in *motheaten* (*me*) and *viable motheaten* (*me*[v]) mice, has on hematopoiesis.

The viability, proliferation, differentiation, and functional activation of hematopoietic cells are regulated by hematopoietic growth factors through interaction with their cognate receptors (2, 18, 20, 32, 36). Although a few hematopoietic growth factor receptors (c-Fms and c-Kit) are receptor tyrosine kinases, the majority of the hematopoietic growth factor receptors belong to a large cytokine receptor superfamily that does not have intrinsic kinase activity. Nevertheless, ligand binding to receptors of the cytokine receptor superfamily rapidly induces tyrosine phosphorylation of cellular substrates as well as one or more of the receptor chains. A critical role for tyrosine phosphorylation in growth regulation has been suggested by the observations that inhibitors of tyrosine kinases inhibit cell growth (56), that inhibitors of tyrosine phosphatases can stimulate cell proliferation in the absence of ligand (47, 54, 57), and that, among mutant receptors, there is a direct correlation between the ability to induce tyrosine phosphorylation and the ability to support ligand-induced cell growth (34, 35, 45).

Although tyrosine phosphorylation has been shown to be an essential component in growth regulation through the cytokine receptors, little is known concerning its regulation. Only recently have the protein tyrosine kinases that associate with the receptors and mediate ligand-inducible tyrosine phosphorylation been identified. Initially it was demonstrated that Lck associates with the interleukin-2 (IL-2) receptor β chain (16, 27, 33), and subsequent studies have implicated other Src family kinases in the responses to granulocyte-macrophage colony-stimulating factor (GM-CSF) (4) and IL-3 (58). However, when these associations have been examined, they have been shown not to be required for mitogenesis. Recently, it has been reported that c-Fes associates with the receptors for GM-CSF (13) and erythropoietin (Epo) (14), but whether this association is required for mitogenesis has not been determined. Lastly, we have recently demonstrated that the Janus kinase 2 (Jak2) associates with the membrane-proximal region of the Epo receptor and is activated following ligand binding (66). The association and activation of kinase activity were shown to be required for mitogenesis. Jak2 has also been implicated in the responses to IL-3 (51), GM-CSF (44), growth hormone (1), and gamma interferon (12, 65), while another Jak family kinase, Tyk2, is required for the response to alpha interferon (61). On the basis of these results, it has been hypothesized that the Jak family of kinases may generally mediate the tyrosine phosphorylation that is associated with ligand binding to receptors of the cytokine receptor superfamily.

In addition to identifying the protein tyrosine kinases, it is evident that protein tyrosine phosphatases (PTPases) will play an essential role in growth regulation. In particular, PTPase inhibitors have been shown to transiently substitute for growth factors and induce a mitogenic response (47, 54, 57). In addition, characteristically, the induction of tyrosine phosphorylation is transient and returns to basal levels within 20 to 30 min (21, 35, 70). The PTPases that mediate these effects have not been identified.

Over the past several years, a variety of PTPases have been identified and molecularly cloned (10). We have identified a PTPase that is preferentially expressed in hematopoietic cells and therefore was termed hematopoietic cell phosphatase (HCP) (68, 69). The same phosphatase was isolated in other studies and was variously termed PTP1C, SHP, or SHPTP1 (30, 43, 48). HCP is a cytoplasmic protein with a tyrosine phosphatase catalytic domain at the carboxyl

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terminus and two contiguous Src homology 2 (SH2) domains at the amino terminus. SH2 domains are found in a variety of signal-transducing proteins and mediate their association with tyrosine-phosphorylated proteins (39). Recently the crystal structure of an SH2 domain associated with a tyrosine-phosphorylated peptide has been described (63, 64). The presence of SH2 domains in HCP suggests a role for this phosphatase in signal transduction through its ability to associate with proteins that are inducibly tyrosine phosphorylated. Consistent with this hypothesis, we have recently demonstrated that HCP associates with c-Kit through the SH2 domains of HCP following ligand binding and receptor phosphorylation (70). A comparable association was not detectable between HCP and c-Fms.

A role for HCP in regulating hematopoiesis has recently been suggested by the consequences of genetic deficiencies of HCP. Recent studies (50, 59) have demonstrated that the *motheaten* (*me*) and *viable motheaten* (me^v) mutations in mice cause either the absence of (*me*) or a reduction in (me^v) HCP activity. Homozygous mice with the *me* and me^v mutations die at 3 and 9 weeks of age, respectively (49). Among the pathological changes are a dramatic increase in the total myeloid precursor cells, including CFU of granulocyte-macrophage cells, CFU of erythroid cells, burst-forming units of erythroid cells, and severe immunodeficiencies affecting B-cell, T-cell and NK-cell differentiation and function. To attempt to obtain a mechanistic explanation for some of these effects, we have explored the possibility that HCP associates with and modifies the function of the IL-3 receptor (IL-3R). The results demonstrate that, following ligand binding, HCP specifically associates with the tyrosine-phosphorylated β chains of the IL-3R through the amino-terminal SH2 domain of HCP. The results also demonstrate that overexpression of HCP in IL-3-dependent cells suppresses growth while inhibition of HCP expression in these cells does not inhibit growth but rather marginally increases the growth response to IL-3. Together the data support the hypothesis that, following IL-3 binding, HCP is recruited to the activated receptor complex, where it dephosphorylates the receptor and potentially other substrates and thereby negatively affects signal transduction and growth.

MATERIALS AND METHODS

Cells and cell culture. DA-3 and transfected DA-3 cells (19, 35) were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and recombinant murine IL-3 (30 U/ml).

Induction of protein tyrosine phosphorylation. Cells were grown in medium supplemented with 0.5% fetal calf serum for 16 h, and stimulation was initiated by adding recombinant hematopoietic growth factors. All stimulations were performed at 37°C. The hematopoietic growth factors used for stimulation included recombinant murine IL-3 (30 U/ml).

Immunoprecipitation and Western blotting (immunoblotting). Antisera against the IL-3R β chain and HCP were developed by immunizing rabbits with keyhole limpet hemocyanin-conjugated peptide corresponding to the C-terminal 15 amino acids of the Aic2A gene product (22) and HCP peptide, as described elsewhere (70). For immunoprecipitation, cells were lysed on ice in cold lysis buffer (50 mM Tris [pH 7.4], 50 mM NaCl, 0.5% sodium deoxycholate, 0.2 mM Na_2VO_4, 20 mM NaF, 1% Nonidet P-40, 2 mM phenylmethylsulfonyl fluoride, 20 μg of aprotinin per ml, and 10% glycerol). Cell lysates were clarified by centrifugation for 20 min at 10,000 × *g* at 4°C. Antibodies were added to cell

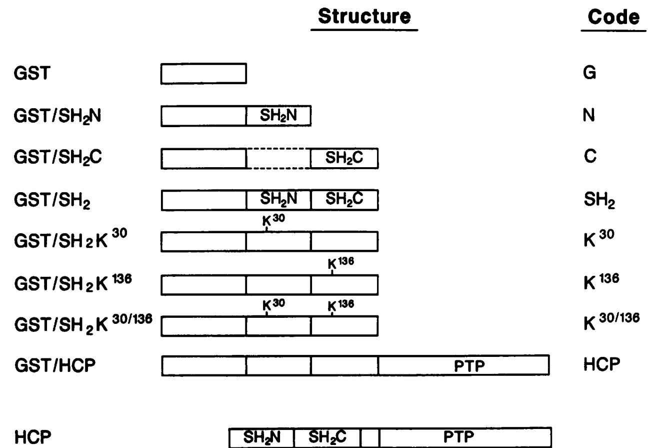


FIG. 1. Construction and preparation of GST fusion proteins of HCP. cDNA fragments encoding different domains of HCP were cloned into pGEX vectors to create GST fusion proteins. Site-specific mutations of Arg-30 to Lys-30 and Arg-136 to Lys-136 in the FLVRE motif of the first and second SH2 domains (SH_2N and SH_2C) are indicated. PTP, PTPase.

lysates, and the mixtures were incubated at 4°C for 60 min with gentle agitation. Immune complexes were collected with protein A-Sepharose 4B (Pharmacia) at 4°C for 30 min and were washed gently in cold lysis buffer twice prior to being boiled off in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) buffer and subjected to SDS-PAGE analysis and Western blotting.

For Western blotting, protein samples, separated on SDS-PAGE gels, were blotted to nitrocellulose membranes (Schleicher & Schuell). The membranes were then blocked with 5% dry milk in washing buffer (10 mM Tris [pH 7.4], 150 mM NaCl, 0.1% Tween 20) for 2 h, incubated with the rabbit polyclonal antibodies (1:3,000 dilution of whole serum) or with the mouse monoclonal antiphosphotyrosine antibody 4G10 (1 μg/ml; UBI) for 2 h, washed for 1 h, incubated with secondary antibodies for 1 h, and then washed for another 1 h. Specific antibody signals were detected with an enhanced chemiluminescence kit (Amersham) or ^{125}I-labeled protein A (ICN) following the manufacturer's procedures. Anti-β-actin monoclonal antibody (Amersham) was also used (1 μg/ml) in Western detection as a control for loading variation of the protein samples.

GST fusion proteins, in vitro binding assay, and PTPase assay. pGEX vectors (Promega) were used to create glutathione *S*-transferase (GST) fusion proteins of HCP. The structures of the various GST-HCP fusion proteins are shown in Fig. 1. Oligonucleotides complementary to murine HCP cDNA sequences were synthesized (Operon) and used as primers in polymerase chain reactions (Cetus) to derive cDNA fragments encoding different regions of HCP or cDNA fragments with site-specific mutations (Arg-30 to Lys-30 and Arg-136 to Lys-136). These fragments were cloned into pGEX vectors and transformed into BL12 *Escherichia coli* bacteria, and then the fusion proteins were purified from isopropyl-β-D-thiogalactopyranoside (IPTG)-induced bacteria with glutathione-Sepharose beads (Pharmacia). For each in vitro assay, glutathione beads, with bound fusion proteins (approximately 4 μg of fusion protein per binding reaction), were incubated in cell lysates at 4°C for 90 min, washed gently four times with cold lysis buffer, boiled off in SDS sample buffer, and analyzed by SDS-PAGE and

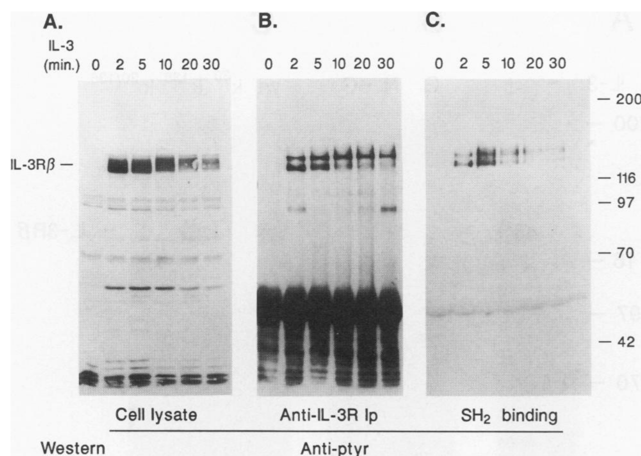


FIG. 2. Association of tyrosine-phosphorylated IL-3R β molecules to GST fusion protein containing the SH2 domains of HCP. Growth factor-deprived DA-3 cells (5×10^6 cells per ml) were stimulated with IL-3 for 0, 2, 5, 10, 20, and 30 min. Cell lysates were prepared and analyzed by immunoprecipitation with antiserum against the IL-3R β subunit as well as by a binding assay with a GST fusion protein containing the two SH2 domains (SH_2). Total cellular proteins (A), proteins precipitated by anti-IL-3R β (B), or cellular proteins bound to GST-SH2 (C) were resolved on SDS-PAGE gels and transferred to membranes, and then the blots were probed with a monoclonal antibody against phosphotyrosine. The migration positions of the IL-3R β subunit and protein molecular size standards (in kilodaltons) are indicated.

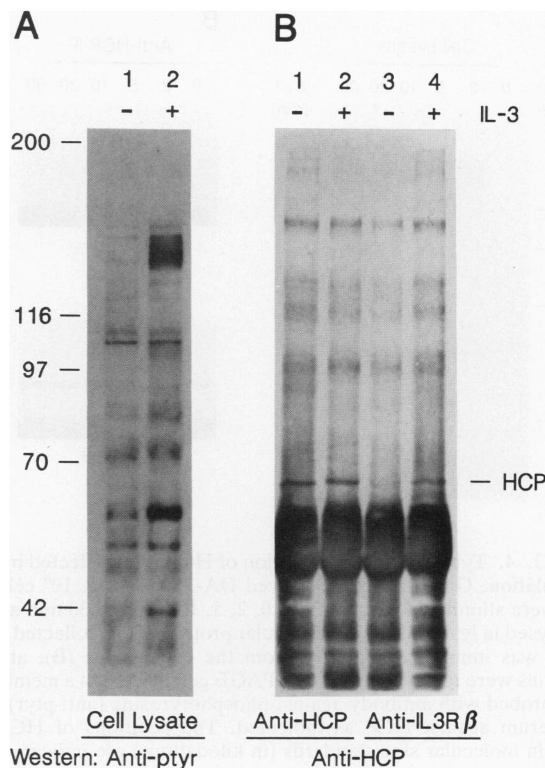


FIG. 3. HCP coimmunoprecipitates with the IL-3R β subunit following IL-3 stimulation. Growth factor-deprived DA-3 cells (5×10^6 cells per reaction) were stimulated with IL-3 for 5 min at 37°C, and the cells were lysed in cold lysis buffer. Total cellular proteins (A) or HCP and IL-3R β proteins, immunoprecipitated from the cell lysates (B), were analyzed by SDS-PAGE and Western blotting. At the top of the panels is indicated whether the protein samples were from unstimulated (−) or IL-3-stimulated (+) cells. The antibodies used for immunoprecipitation and Western detection are shown beneath each panel. The positions of protein molecular size standards (in kilodaltons) and HCP are indicated. Anti-ptyr, antibody against phosphotyrosine.

Western blotting. Approximately 5×10^6 cells were used in each binding reaction mixture.

For PTPase assays, the IL-3R β subunit was immunoprecipitated from DA-3 cells stimulated with IL-3 and incubated with 1 μg of GST or GST-HCP fusion proteins in PTPase buffer {25 mM PIPES [piperazine-*N*,*N'*-bis(2-ethanesulfonic acid)], pH 7.0; 5 mM EDTA; and 20 mM dithiothreitol} for 10 min at 37°C. The reactions were terminated by adding 2× SDS-PAGE sample buffer and boiling for 5 min. The phosphotyrosine content of the IL-3R β proteins was determined by SDS-PAGE and Western detection with monoclonal antibody against phosphotyrosine as described previously (70).

Construction of inducible expression plasmids for HCP transfection and cell proliferation assays. The plasmids pMAMneo/HCP/S and pMAMneo/HCP/A were constructed by inserting the murine HCP cDNA clone into the *Eco*RI site of plasmid pMAMneo (Promega) in sense and antisense orientations, respectively (see Fig. 7A). The inserted HCP cDNA clone was expressed under the control of the mouse mammary tumor virus long terminal repeat promoter, which is inducible by dexamethasone (Dex) (46). The plasmid DNAs were transfected into DA-3 cells by electroporation (35). Transfected cells were selected in medium containing G418 (0.5 mg/ml) and murine IL-3 (200 U/ml). Several subclones for each plasmid were isolated by limited dilution and analyzed for the expression of HCP in the absence or presence of 5 μM Dex (Sigma). The clones that showed the highest inducible HCP expression were selected for each plasmid and used for further characterization. The proliferation of DA-3 transfectants in response to IL-3 (5 U/ml) in the absence or presence of Dex (5 μM) was determined by counting cells every 24 h following the initial plating of 2×10^4 cells per ml.

RESULTS

HCP associates with the IL-3R β subunit following ligand binding. Our previous studies demonstrated that HCP transiently associates with the c-Kit receptor kinase following ligand stimulation but not with the c-Fms receptor (70). The association with c-Kit occurs through the SH2 domain of HCP and requires tyrosine phosphorylation of the c-Kit protein. Although the receptors for most of the hematopoietic growth factors do not have intrinsic kinase activity, they are rapidly tyrosine phosphorylated following ligand binding. Therefore it was important to examine the ability of HCP to associate with these receptors. Initially we examined the potential association of HCP with the IL-3R following IL-3 stimulation of the murine IL-3-dependent myeloid cell line DA-3 (19). The murine IL-3R is a heterodimer that consists of an IL-3-specific 70-kDa α subunit and one of two alternative 130- to 140-kDa β subunits termed Aic2A and Aic2B. The Aic2A subunit also associates with GM-CSF- and IL-5-specific α subunits (17, 22, 25, 55). Both β chains are inducibly tyrosine phosphorylated following ligand binding (8, 15, 38, 52). Stimulation of DA-3 cells with IL-3 induces a rapid, but transient, tyrosine phosphorylation of several proteins (Fig. 2A), including two major proteins of

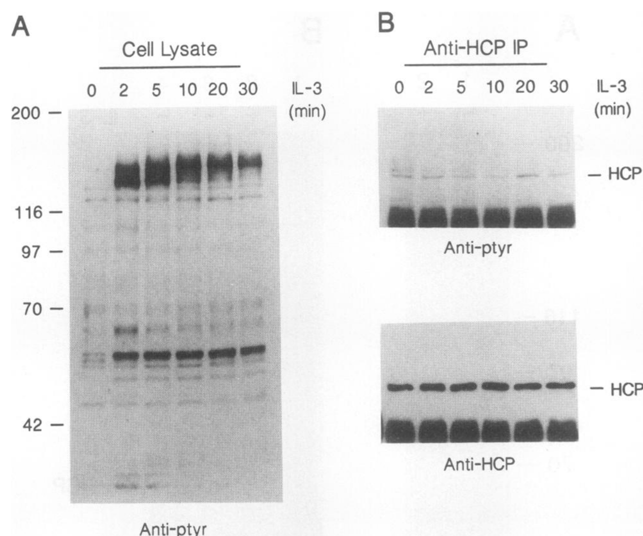


FIG. 4. Tyrosine phosphorylation of HCP is not affected by IL-3 stimulation. Growth factor-deprived DA-3 cells (5×10^6 cells per ml) were stimulated with IL-3 for 0, 2, 5, 10, 20, and 30 min at 37°C and lysed in lysis buffer. Total cellular proteins were collected (A) or HCP was immunoprecipitated from the cell lysates (B), and the proteins were resolved in an SDS-PAGE gel, blotted to a membrane, and probed with antibody against phosphotyrosine (anti-ptyr) or an antiserum against HCP as indicated. The positions of HCP and protein molecular size standards (in kilodaltons) are indicated.

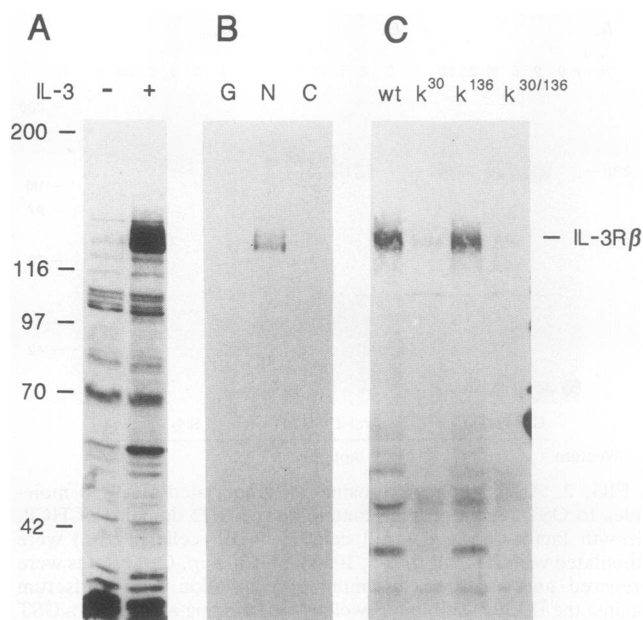


FIG. 5. GST fusion proteins of the first SH2 domain (SH2N) of HCP specifically bind to tyrosine-phosphorylated IL-3R β. Growth factor-deprived DA-3 cells were stimulated with IL-3 for 5 min, and cell lysates were prepared and analyzed by binding assays with GST fusion proteins containing the SH2 domains of HCP. SDS-PAGE and Western blotting were used to analyze total cellular proteins from unstimulated (−) and IL-3-stimulated (+) cells (A); proteins of IL-3-stimulated cells that bound to GST (G) or GST fusion proteins containing the first (N) or the second (C) SH2 domain (B); and GST fusion proteins containing the SH2 domain (wt) or GST-SH2 fusion proteins with site-specific mutations of both Arg-30 and Arg-136 to Lys ($k^{30/136}$), Arg-30 to Lys (k^{30}), and Arg-136 to Lys (k^{136}) (C). The Western blots were detected with antibody against phosphotyrosine. The positions of IL-3R β chain and protein molecular size standards (in kilodaltons) are indicated.

130 to 140 and 60 kDa. The phosphoproteins of 130 to 140 kDa were immunoprecipitated by antibodies to the IL-3R β subunit (Fig. 2B). The 90-kDa, coimmunoprecipitating phosphoprotein seen in Fig. 2B is seen irregularly; thus its significance is unclear. To determine whether any of these phosphoproteins were bound by HCP, extracts were incubated with GST fusion protein containing the two SH2 domains of HCP. The bound proteins were then isolated and resolved on SDS-PAGE gels, and the blots were probed with a monoclonal antibody against phosphotyrosine. As shown (Fig. 2C), the 130- to 140-kDa proteins were specifically bound by the SH2 domains of HCP, suggesting that HCP associates with the IL-3R β subunits.

To further determine whether HCP associates with the IL-3R β subunits, lysates from unstimulated DA-3 cells or cells stimulated with IL-3 were incubated with antibodies against the IL-3R β subunit. The immunoprecipitates were then resolved by SDS-PAGE, and the blots were probed with an antiserum against HCP. As illustrated (Fig. 3B, lanes 3 and 4), HCP was detected in immunoprecipitates of IL-3R β from stimulated but not from unstimulated cells, demonstrating that HCP associates with the IL-3R β subunits following ligand binding.

Previous studies have shown that tyrosine phosphatases are inducibly tyrosine phosphorylated following ligand stimulation (9, 62, 67). It was therefore important to determine whether HCP was inducibly tyrosine phosphorylated in DA-3 cells following IL-3 stimulation. As shown (Fig. 4B), there is an apparent, low level of tyrosine phosphorylation of HCP in DA-3 cells, and this level did not alter over the times examined following IL-3 stimulation. For controls, the changes seen in the phosphotyrosine-containing proteins in total cell lysates are shown (Fig. 4A), and the levels of immunoprecipitable HCP were determined (Fig. 4B). We have also failed to detect any changes in in vitro HCP activity following IL-3 stimulation (data not shown).

The amino-terminal SH2 domain of HCP mediates its association with the IL-3R β subunit. To determine the domain of HCP that mediates its association with the IL-3R β subunit, GST protein and GST fusion proteins containing the amino-terminal SH2 domain (N) or the second SH2 domain (C) of HCP were prepared and incubated with lysates of DA-3 cells stimulated with IL-3 (Fig. 5B). The induction of tyrosine phosphorylation observed in these experiments is also shown (Fig. 5A). As illustrated, only the fusion protein containing the amino-terminal SH2 domain was able to bind the tyrosine-phosphorylated IL-3R β subunit. Previous studies (31) have shown that replacement of the highly conserved Arg residue with a Lys in the SH2 FLVRE motif in c-Abl can abrogate its binding to phosphotyrosine-containing proteins. We therefore made comparable substitutions in one or both of the SH2 domains of HCP (Arg-30 to Lys and Arg-136 to Lys) and prepared GST fusion proteins of these mutants. In in vitro binding assays (Fig. 5C), the GST fusion protein containing the mutation in the second SH2 domain bound the tyrosine-phosphorylated IL-3R β subunit as efficiently as the wild-type fusion protein did. In contrast, mutation of the amino-terminal SH2 domain, or mutation of both SH2 domains, abrogated binding. Therefore, HCP associates with the tyrosine-phosphorylated IL-3R β chain through its amino-terminal SH2 domain.

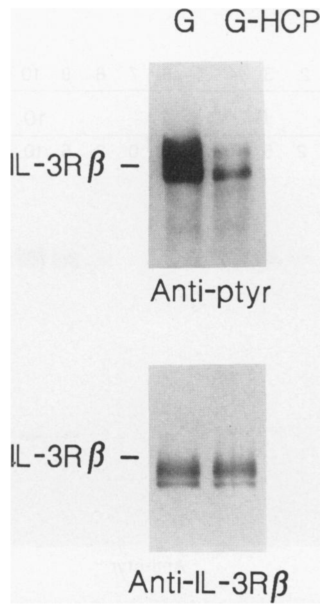


FIG. 6. GST-HCP fusion protein dephosphorylates phosphotyrosine IL-3R β chain in vitro. The IL-3R β chain was immunoprecipitated from DA-3 cells which had been stimulated with IL-3. The immunoprecipitates were incubated in the presence of GST protein (lane G) or GST-HCP fusion protein (lane G-HCP) and then analyzed by SDS-PAGE and Western blotting. The blots were probed with monoclonal antibody against phosphotyrosine (anti-ptyr) or anti-IL-3R β antiserum as indicated. The position of migration of the IL-3R β subunit is indicated.

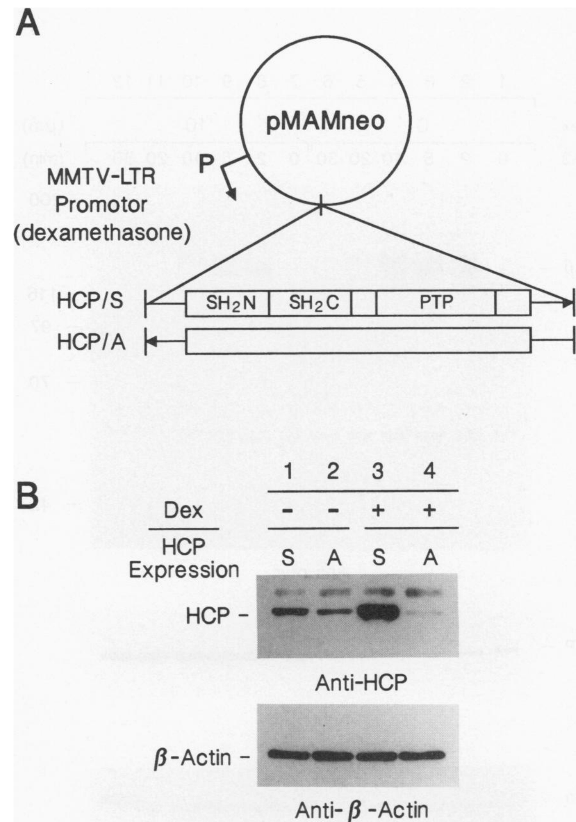
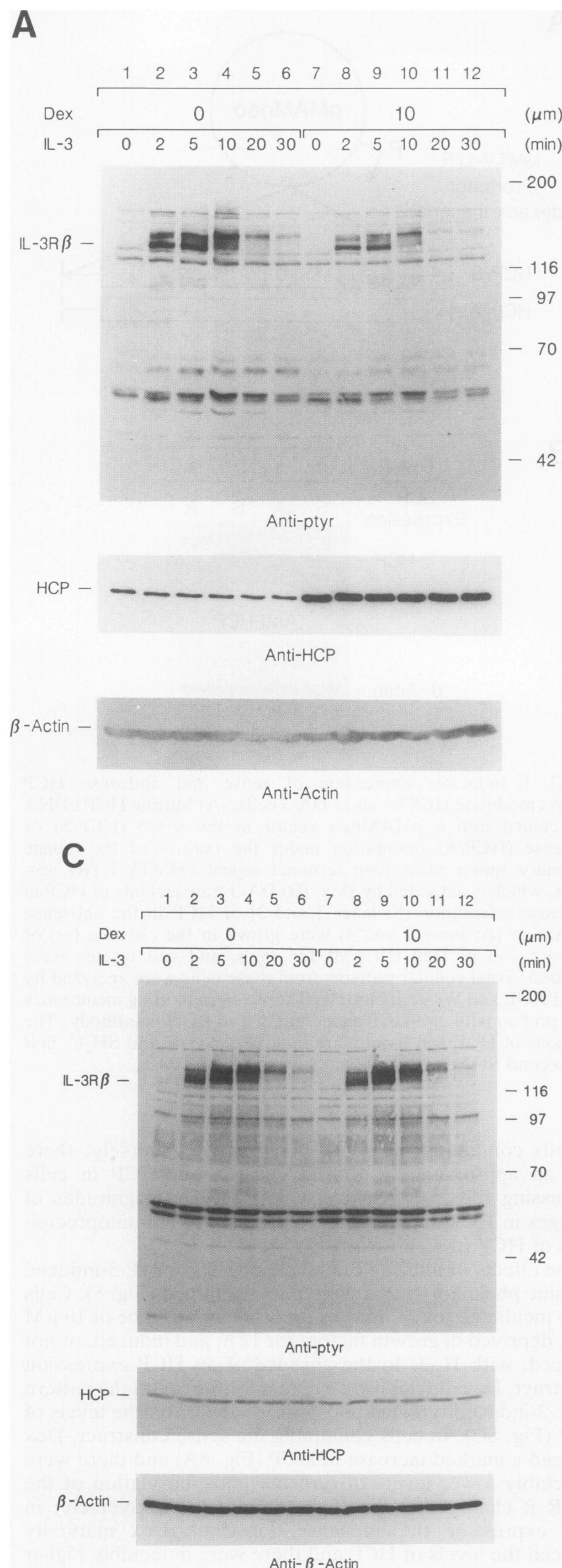


FIG. 7. Inducible expression of sense and antisense HCP cDNAs modulate HCP levels in DA-3 cells. (A) Murine HCP cDNA was cloned into a pMAMneo vector in the sense (HCP/S) or antisense (HCP/A) orientation under the control of the mouse mammary tumor virus long terminal repeat (MMTV-LTR) promoter, which is activated by Dex. (B) DA-3 transfectants of HCP in the sense orientation (S; lanes 1 and 3) or HCP in the antisense orientation (A; lanes 2 and 4) were grown in the absence (−) or presence (+) of Dex (10 μM) for 48 h, and cell lysates were prepared. Total cellular proteins from these cells were analyzed by SDS-PAGE and Western blotting. The Western blotting membranes were probed with anti-HCP antiserum or anti-β-actin antibody. The positions of HCP and β-actin are indicated. SH_2N and SH_2C, first and second SH2 domains, respectively; PTP, PTPase.

The ability to associate with the IL-3R β chain suggested the possibility that the receptor is a substrate for HCP. To assess this, cells were stimulated with IL-3 for 10 min and the tyrosine-phosphorylated IL-3R β chain was immunoprecipitated. The immunoprecipitates were subsequently incubated with GST or a GST fusion protein containing the entire HCP protein. Following incubation, the proteins were resolved by SDS-PAGE, blotted to nitrocellulose, and probed with an antiserum against the IL-3R β chain or a monoclonal antibody against phosphotyrosine. As illustrated (Fig. 6), incubation with the GST-HCP fusion protein resulted in the loss of reactivity of the IL-3R β chain with the monoclonal antibody against phosphotyrosine but had no effect on the reactivity with the antiserum against the receptor. Therefore, HCP is capable of dephosphorylating the β chain in in vitro reactions.

HCP down-regulates IL-3-induced tyrosine phosphorylation and proliferation. The association of HCP with the IL-3R β chain following ligand stimulation suggested a potential functional role in the IL-3 response. To manipulate HCP expression, we obtained clones of DA-3 cells into which we had introduced the HCP cDNA, in sense and antisense orientations, under control of the mouse mammary tumor virus long terminal repeat promoter (Fig. 7A). The effects of Dex on the levels of HCP are shown in Fig. 7B. In the absence of Dex, the levels of HCP protein were similar, although the levels in cells containing the antisense construct were consistently slightly lower than those in cells containing the construct in the sense orientation. However, when the cells were cultured in the presence of 10 μM Dex for 48 h, there was a marked effect on the levels of HCP. As shown, there was an approximately 10-fold increase in HCP in cells containing the sense construct. Conversely, there was an approximately 10-fold decrease in HCP in cells expressing the antisense construct. Similar magnitudes of changes in HCP activity were detectable in immunoprecipitates of HCP (data not shown).

The effects of altering the levels of HCP on IL-3-induced tyrosine phosphorylation were next examined (Fig. 8). Cells were incubated for 24 h in the presence or absence of 10 μM Dex, deprived of growth factors for 12 h, and induced, or not induced, with IL-3. In the absence of an HCP expression construct, Dex did not have a detectable effect on the pattern of IL-3-induced tyrosine phosphorylation or on the levels of HCP (Fig. 8C). In cells containing the sense construct, Dex induced a marked increase in HCP (Fig. 8A) and there were detectably lower levels of tyrosine phosphorylation of the IL-3R β chain following IL-3 stimulation. Conversely, in cells expressing the antisense construct, Dex markedly reduced the levels of HCP and there were detectably higher levels of tyrosine phosphorylation of the IL-3R β chain



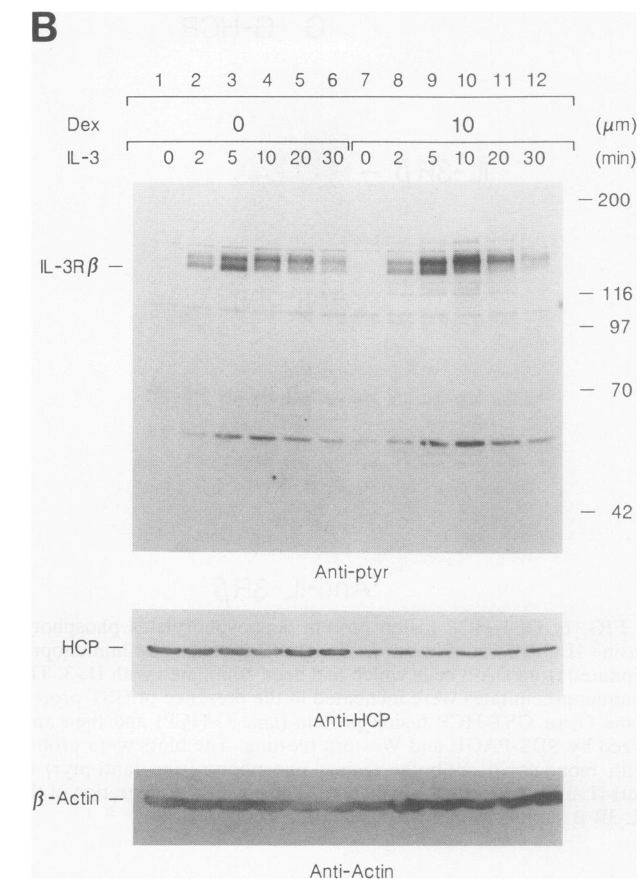


FIG. 8. Comparison of IL-3-induced tyrosine phosphorylation and proliferation of DA-3 transfectants. DA-3 transfectants of HCP in the sense orientation (A), HCP in the antisense orientation (B), and pMAMneo (C) were grown in the absence or presence of 10 μM Dex for 24 h in medium supplemented with IL-3 for the initial 12 h and deprived of growth factor for the remaining 12 h. The cells were stimulated with IL-3 for 0, 2, 5, 10, 20, and 30 min, and cell lysates were prepared at each time point. Total cellular proteins were analyzed by SDS-PAGE and Western blotting. The Western blots were probed with antibody against phosphotyrosine (anti-ptyr), anti-HCP antiserum, and anti-β-antibody (anti-actin). The positions of IL-3R β, HCP, β-actin and protein molecular size standards (in kilodaltons) are indicated.

following IL-3 stimulation. In addition, the presence of the HCP expression constructs also affected the levels of tyrosine phosphorylation of other cellular substrates, although the somewhat-more-variable detection of these proteins precludes a definitive conclusion.

The effects of altering HCP levels on cellular proliferation are shown in Fig. 9A. In the absence of Dex (Fig. 9A, HCP/A− and HCP/S−), there were only slight differences in the cell numbers at 2 or 3 days following initiation of the cultures. However, in the presence of Dex there were dramatic differences in growth rates. The numbers of cells containing the antisense construct which were cultured with Dex were increased at days 2 and 3 relative to those which were cultured in the absence of Dex. More dramatically, the growth of cells containing the sense construct was markedly reduced in the presence of Dex. In contrast, Dex had no effect on the growth of the parental cells (Fig. 9B). These results demonstrate that HCP negatively regulates the growth of IL-3-dependent cells.

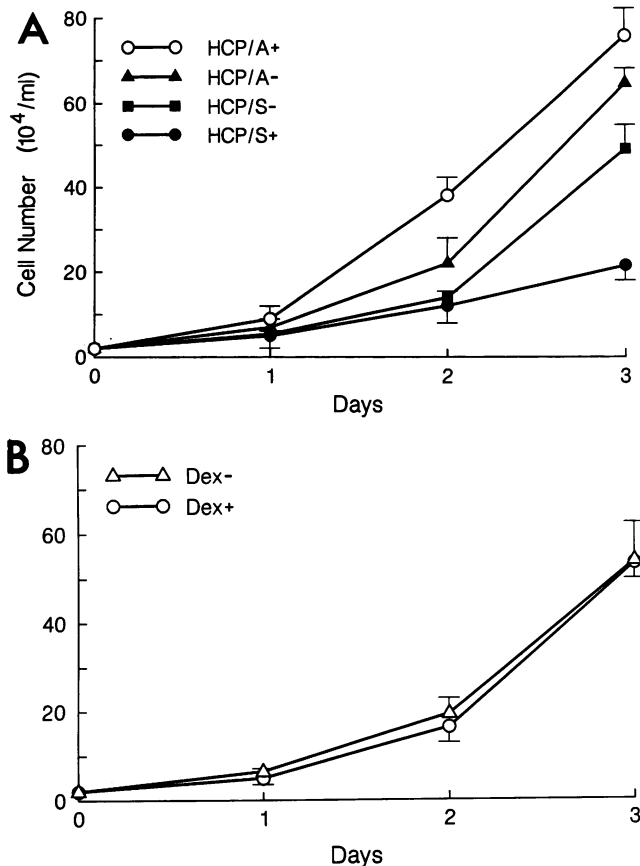


FIG. 9. Increased expression of HCP suppresses IL-3-induced proliferation. Stable cell clones isolated from DA-3 cells transfected with the indicated constructs (A) and parental DA-3 cells (B) were collected by centrifugation, resuspended in fresh medium, and grown in the absence (−) and presence (+) of Dex (5 μM) in medium supplemented with IL-3. The cell numbers were determined by cell counting over a 3-day period. Each datum point is the average of the results of two separate experiments, and error bars indicate standard deviations. HCP/A, DA-3 transfectants of HCP in the antisense orientation; HCP/S, DA-3 transfectants of HCP in the sense orientation. (B) Numbers along the *y* axis indicate cells (10^4) per milliliter.

DISCUSSION

The regulation of protein tyrosine phosphorylation is hypothesized to play a critical role in growth regulation of cells of a variety of lineages, including IL-3-dependent hematopoietic cells. In support of this hypothesis, activated tyrosine kinases can abrogate the requirement of cells for IL-3 (3, 24, 29, 41); IL-3 induces a rapid, but transient, increase in protein tyrosine phosphorylation (21, 37, 52, 53); and inhibitors of tyrosine phosphatases can partially replace the requirements for growth factors (47, 54, 57). The studies presented here explored the potential role of the PTPase HCP in growth regulation of IL-3-dependent cells and demonstrate that HCP associates with the tyrosine-phosphorylated IL-3R and down-regulates the response to IL-3.

A potential role for HCP in growth regulation was initially suggested by the presence of SH2 domains. SH2 domains are present in a variety of molecules that have been implicated in signal transduction and mediate their association with receptor protein tyrosine kinases, including c-Fms, c-Kit, platelet-derived growth factor receptor, and epidermal growth factor receptor (39). More recently, the SH2 domain of phosphatidylinositol 3-kinase has been shown to mediate its association with the tyrosine-phosphorylated receptors of the cytokine superfamily, including the Epo receptor (5) and the IL-4R (23). Therefore, both receptor kinases and the cytokine receptor superfamily members may utilize comparable biochemical means to recruit signal-transducing molecules into active receptor complexes.

HCP associates with the IL-3R β chain through the amino-terminal SH2 domain. This was demonstrated by the ability of the isolated SH2 domain to bind as well as by the ability of mutations in the FLVRE motif to abrogate binding. Importantly, a comparable mutation in the second SH2 domain did not affect binding. Our previous studies (70) demonstrated that the amino-terminal SH2 domain is also involved in the association of HCP with c-Kit. Recent studies of the structures of SH2 domains interacting with peptides have indicated that an SH2 domain interacts with a region of 6 to 10 amino acids flanking the target phosphotyrosine (64). In screening the structures of the IL-3R β chain and c-Kit, we were not able to identify a tyrosine residue with similar flanking amino acids that might predict the binding sites. Studies are currently directed to identifying the binding sites through mutational analysis.

The role of tyrosine phosphatases in growth regulation could be envisioned to be either positive or negative. Inhibitor studies have suggested a negative role for PTPases in the growth regulation of hematopoietic cells (47, 54, 57). However, in T lymphoid cells, the lack of CD45, a receptor PTPase, is associated with an inability to respond through the T-cell antigen receptor in either proliferation or protein tyrosine phosphorylation (28, 42), suggesting that CD45 plays a positive regulatory role. This has been hypothesized to involve the removal of phosphates from negative-regulatory sites on Src family kinases (26).

Recent studies have suggested that a phosphatase that is closely related to HCP is also positively involved in growth regulation. In particular, several groups have cloned a PTPase, containing two amino-terminal SH2 domains, which is variously termed Syp, PTP 1D, and SH-PTP2 (9, 11, 62). Syp is more widely expressed than HCP and is inducibly tyrosine phosphorylated in the response to platelet-derived growth factor or epidermal growth factor. Tyrosine phosphorylation of Syp activates its phosphatase activity and is hypothesized to function as a positive signalling component by dephosphorylation of phosphotyrosines that negatively regulate the signalling potential of proteins such as the Src kinases, comparable to the model proposed for CD45. In support of a positive role in growth regulation, the *Drosophila* homolog of Syp, *corkscrew*, functions downstream of the Torso receptor tyrosine kinase in a positive manner (40).

In contrast to Syp, HCP can be hypothesized to be essential for the down-regulation of growth signals in hematopoietic cells. This hypothesis is most strongly supported by the phenotype of mutant mice that genetically lack or are deficient in HCP (49, 50, 60). The *motheaten* (*me*) and *viable motheaten* (me^v) mutations were initially characterized as contributing to immunodeficiency; however, a variety of hematopoietic lineages have been subsequently shown to be affected. The mutations are associated with CSF-1-independent proliferation of macrophages and increased sensitivity of CFU of erythroid cells to Epo. There is also a dramatic increase in the numbers of erythroid precursors in the spleens, consistent with an effect on cells at a stage of differentiation which is normally regulated by the levels of IL-3. Thus the phenotypes of *me* and me^v mice suggest that

HCP negatively regulates a number of hematopoietic lineages.

A general role for HCP as a negative regulator in hematopoiesis is supported by the observation that it associates with several receptors. In addition to the IL-3R complex, we have observed association of HCP with the GM-CSF receptor, which shares a common β subunit with the IL-3R (17, 22, 36, 71). This further suggests that HCP can associate with the IL-5R complex, which also utilizes the same, shared β subunit. We have previously shown that HCP associates with c-Kit (70), although, interestingly, association with the c-Fms was not detected under comparable conditions. It should be noted, however, that it has been reported that CSF-1 induces tyrosine phosphorylation of HCP (67). More recently we have found that HCP also associates with the tyrosine-phosphorylated Epo receptor (72) in a region of the receptor that has been shown to negatively affect the response to Epo (6). This region, when deleted by mutations in humans, causes erythrocytosis (7). Since the phosphorylation of one or more chains of receptors is commonly observed among the cytokine receptors, it will be of interest to examine other receptors for their ability to associate with HCP.

In summary, the data support the hypothesis that HCP is essential for the down-regulation of the response of hematopoietic cells to growth factors. As a consequence of growth factor stimulation, the individual receptors are inducibly tyrosine phosphorylated. The receptors are then capable of recruiting HCP to the active receptor complex, where it can dephosphorylate the receptor as well as other substrates for tyrosine phosphorylation that have been recruited to the complex. In addition, HCP may dephosphorylate and thereby inactivate Jak2 kinase. These reactions ensure that in the absence of continual growth factor binding, the receptor complexes are not constitutively activated. The extent to which HCP plays a comparable role in the lymphoid lineages is currently being evaluated.

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REFERENCES

1. **Artgetsinger, L. S., G. S. Campbell, X. Yang, B. A. Witthuhn, O. Silvennoinen, J. N. Ihle, and C. Carter-Su.** 1993. Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. Cell **74:**237–244.
2. **Clark, S. C., and R. Kamen.** 1987. The human hematopoietic colony-stimulating factors. Science **236:**1229–1237.
3. **Cleveland, J. L., M. Dean, N. Rosenberg, J. Y. Wang, and U. R. Rapp.** 1989. Tyrosine kinase oncogenes abrogate interleukin-3 dependence of murine myeloid cells through signalling pathways involving c-*myc*: conditional regulation of c-*myc* transcription by temperature-sensitive v-*abl*. Mol. Cell. Biol. **9:**5685–5695.
4. **Corey, S., A. Egulinoa, K. Puyana-Theall, J. B. Bolen, L. Cantley, F. Mollinedo, T. R. Jackson, P. T. Hawkins, and L. R. Stephens.** 1993. Granulocyte macrophage-colony stimulating factor stimulates both association and activation of phosphoinositide 3OH-kinase and *src*-related tyrosine kinase(s) in human myeloid derived cells. EMBO J. **12:**2681–2690.
5. **Damen, J. E., A. L.-F. Mui, L. Puil, T. Pawson, and G. Krystal.** 1993. Phosphatidylinositol 3-kinase associates, via its src homology 2 domains, with the activated erythropoietin receptor. Blood **81:**3204–3210.
6. **D'Andrea, A. D., A. Yoshimura, H. Youssoufian, L. I. Zon, J. W. Koo, and H. F. Lodish.** 1991. The cytoplasmic region of the erythropoietin receptor contains nonoverlapping positive and negative growth-regulatory domains. Mol. Cell. Biol. **11:** 1980–1987.
7. **De La Chapelle, A., A. Traskelin, and E. Juvonen.** 1993. Truncated erythropoietin receptor causes dominantly inherited benign human erythrocytosis. Proc. Natl. Acad. Sci. USA **90:** 4495–4499.
8. **Duronio, V., I. Clark-Lewis, B. Federsppiel, J. S. Wieler, and J. W. Schrader.** 1992. Tyrosine phosphorylation of receptor beta subunits and common substrates in response to interleukin-3 and granulocyte-macrophage colony-stimulating factor. J. Biol. Chem. **267:**21856–21863.
9. **Feng, G., C. Hui, and T. Pawson.** 1993. SH2-containing phosphotyrosine phosphatase as a target of protein-tyrosine kinases. Science **259:**1607–1611.
10. **Fischer, E. H., H. Charbonneau, and N. K. Tonks.** 1991. Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. Science **253:**401–406.
11. **Freeman, R. M. J., J. Plutzky, and B. G. Neel.** 1992. Identification of a human src homology 2-containing protein-tyrosine-phosphatase: a putative homolog of *Drosophila* corkscrew. Proc. Natl. Acad. Sci. USA **89:**11239–11243.
12. **Guschin, D., D. Watling, B. A. Witthuhn, F. W. Quelle, N. C. Rogers, O. Silvennoinen, C. Schindler, G. R. Stark, J. N. Ihle, and I. M. Kerr.** Activation of the protein tyrosine kinase Jak2 in response to interferon-τ. Submitted for publication.
13. **Hanazono, Y., S. Chiba, K. Sasaki, H. Mano, A. Miyajima, K. Arai, Y. Yazaki, and H. Hirai.** 1993. c-fps/fes protein-tyrosine kinase is implicated in a signaling pathway triggered by granulocyte-macrophage colony-stimulating factor and interleukin-3. EMBO J. **12:**1641–1646.
14. **Hanazono, Y., S. Chiba, K. Sasaki, H. Mano, Y. Yazaki, and H. Hirai.** 1993. Erythropoietin induces tyrosine phosphorylation and kinase activity of the *c-fps/fes* proto-oncogene product in human erythropoietin-responsive cells. Blood **81:**3193–3196.
15. **Hara, T., and A. Miyajima.** 1992. Two distinct functional high affinity receptors for mouse interleukin-3 (IL-3). EMBO J. **11:**1875–1884.
16. **Hatakeyama, M., T. Kono, N. Kobayashi, A. Kawahara, S. D. Levin, R. M. Perlmutter, and T. Taniguchi.** 1991. Interaction of the IL-2 receptor with the src-family kinase $p56^{lck}$: identification of novel intermolecular association. Science **252:**1523–1528.
17. **Hayashida, K., T. Kitamura, D. M. Gorman, K. Arai, T. Yokota, and A. Miyajima.** 1990. Molecular cloning of a second subunit of the receptor for human granulocyte-macrophage colony-stimulating factor (GM-CSF): reconstitution of a high-affinity GM-CSF receptor. Proc. Natl. Acad. Sci. USA **87:**9655–9659.
18. **Ihle, J. N.** 1992. Interleukin-3 and hematopoiesis, p. 65–106. *In* T. Kishimoto (ed.), Interleukins: molecular biology and immunology. Karger, Basel.
19. **Ihle, J. N., and D. Askew.** 1989. Origins and properties of hematopoietic growth factor-dependent cell lines. Int. J. Cell. Cloning **1:**1–30.
20. **Ihle, J. N., F. W. Quelle, and O. Miura.** Signal transduction through the receptor for erythropoietin. Semin. Immunol., in press.
21. **Isfort, R., R. Abraham, R. D. Huhn, A. R. Frackelton, Jr., and J. N. Ihle.** 1988. Stimulation of factor-dependent myeloid cell lines with interleukin 3 induces the tyrosine phosphorylation of several cellular substrates. J. Biol. Chem. **263:**19203–19209.
22. **Itoh, N., S. Yonehara, J. Schreurs, D. M. Gorman, K. Maruyama, A. Ishii, I. Yahara, K. I. Arai, and A. Miyajima.** 1990. Cloning of an interleukin-3 receptor gene: a member of a distinct receptor gene family. Science **247:**324–327.
23. **Izuhara, K., and N. Harada.** 1993. Interleukin-4 (IL-4) induces protein tyrosine phosphorylation of the IL-4 receptor and association of phosphatidylinositol 3-kinase to the IL-4 receptor in a

mouse T cell line, HT2. J. Biol. Chem. **268**:13097–13102.

24. **Kipreos, E. T., and J. Y. J. Wang.** 1988. Reversible dependence on growth factor interleukin-3 in myeloid cells expressing temperature sensitive v-abl oncogene. Oncogene Res. **2**:277–284.
25. **Kitamura, T., N. Sato, K. Arai, and A. Miyajima.** 1991. Expression cloning of the human IL-3 receptor cDNA reveals a shared beta subunit for the human IL-3 and GM-CSF receptors. Cell **66**:1165–1174.
26. **Klausner, R. D., and L. E. Samelson.** 1991. T cell antigen receptor activation pathways: the tyrosine kinase connection. Cell **64**:875–878.
27. **Kobayashi, N., T. Kono, M. Hatakeyama, Y. Minami, T. Miyazaki, R. M. Perlmutter, and T. Taniguchi.** 1993. Functional coupling of the *src*-family protein tyrosine kinases $p59^{fyn}$ and $p53/56^{lyn}$ with the interleukin 2 receptor: implications for redundancy and pleiotropism in cytokine signal transduction. Proc. Natl. Acad. Sci. USA **90**:4201–4205.
28. **Koretzky, G. A., J. Picus, T. Schultz, and A. Weiss.** 1991. Tyrosine phosphatase CD45 is required for T-cell antigen receptor and CD2-mediated activation of a protein tyrosine kinase and interleukin 2 production. Proc. Natl. Acad. Sci. USA **88**:2037–2041.
29. **Mathey-Prevot, B., G. Nabel, R. Palacios, and D. Baltimore.** 1986. Abelson virus abrogation of interleukin-3 dependence in a lymphoid cell line. Mol. Cell. Biol. **6**:4133–4135.
30. **Matthews, R. J., D. B. Bowne, E. Flores, and M. L. Thomas.** 1992. Characterization of hematopoietic intracellular protein tyrosine phosphatases: description of a phosphatase containing an SH2 domain and another enriched in proline-, glutamic acid-, serine-, and threonine-rich sequences. Mol. Cell. Biol. **12**:2396–2405.
31. **Mayer, B. J., P. K. Jackson, R. A. Van Etten, and D. Baltimore.** 1992. Point mutations in the *abl* SH2 domain coordinately impair phosphotyrosine binding in vitro and transforming activity in vivo. Mol. Cell. Biol. **12**:609–618.
32. **Metcalf, D.** 1989. The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. Nature (London) **339**:27–30.
33. **Minami, Y., T. Kono, K. Yamada, N. Kobayashi, A. Kawahara, R. M. Perlmutter, and T. Taniguchi.** 1993. Association of $p56^{lck}$ with IL-2 receptor β chain is critical for the IL-2-induced activation of $p56^{lck}$. EMBO J. **12**:759–768.
34. **Miura, O., J. L. Cleveland, and J. N. Ihle.** 1993. Inactivation of erythropoietin receptor function by point mutations in a region having homology with other cytokine receptors. Mol. Cell. Biol. **13**:1788–1795.
35. **Miura, O., A. D'Andrea, D. Kabat, and J. N. Ihle.** 1991. Induction of tyrosine phosphorylation by the erythropoietin receptor correlates with mitogenesis. Mol. Cell. Biol. **11**:4895–4902.
36. **Miyajima, A., T. Kitamura, N. Harada, T. Yokota, and K. Arai.** 1992. Cytokine receptors and signal transduction. Annu. Rev. Immunol. **10**:295–331.
37. **Morla, A. O., J. Schreurs, A. Miyajima, and J. W. J. Wang.** 1988. Hematopoietic growth factors activate the tyrosine phosphorylation of distinct sets of proteins in interleukin-3-dependent murine cell lines. Mol. Cell. Biol. **8**:2214–2218.
38. **Mui, A. L. F., R. J. Kay, R. K. Humphries, and G. Krystal.** 1992. Ligand-induced phosphorylation of the murine interleukin 3 receptor signals its cleavage. Proc. Natl. Acad. Sci. USA **89**:10812–10816.
39. **Pawson, T., and G. D. Gish.** 1992. SH2 and SH3 domains: from structure to function. Cell **71**:359–362.
40. **Perkins, L. A., I. Larsen, and N. Perrimon.** 1992. Corkscrew encodes a putative protein tyrosine phosphatase that functions to transduce the terminal signal from the receptor tyrosine kinase torso. Cell **70**:225–236.
41. **Pierce, J. H., P. P. Di Fiore, S. A. Aaronson, M. Potter, J. Pumphrey, A. Scott, and J. N. Ihle.** 1985. Neoplastic transformation of mast cells by Abelson-MuLV: abrogation of IL-3 dependence by a nonautocrine mechanism. Cell **41**:685–693.
42. **Pingel, J. T., and M. L. Thomas.** 1989. Evidence that the leukocyte-common antigen is required for antigen-induced T lymphocyte proliferation. Cell **58**:1055–1065.
43. **Plutzky, J., B. G. Neel, and R. D. Rosenberg.** 1992. Isolation of a src homology 2-containing tyrosine phosphatase. Proc. Natl. Acad. Sci. USA **89**:1123–1127.
44. **Quelle, F. W., B. A. Witthuhn, and J. N. Ihle.** Unpublished data.
45. **Sakamaki, K., I. Miyajima, T. Kitamura, and A. Miyajima.** 1992. Critical cytoplasmic domains of the common β subunit of the human GM-CSF, IL-3 and IL-5 receptors for growth signal transduction and tyrosine phosphorylation. EMBO J. **11**:3541–3549.
46. **Sardet, C., A. Franchi, and J. Pouyssegur.** 1989. Molecular cloning, primary structure and expression of the human growth factor-activatable Na+/H+ antiporter. Cell **56**:271–280.
47. **Secrist, J. P., L. A. Burns, L. Karnitz, G. A. Loretzky, and R. T. Abraham.** 1993. Stimulatory effects of the protein tyrosine inhibitor, pervanidate, on T-cell activation events. J. Biol. Chem. **268**:5886–5893.
48. **Shen, S. H., L. Bastien, B. I. Posner, and P. Chrëtien.** 1991. A protein-tyrosine phosphatase with sequence similarity to the SH2 domain of the protein-tyrosine kinases. Nature (London) **352**:736–739.
49. **Shultz, L. D.** 1991. Hematopoiesis and models of immunodeficiency. Semin. Immunol. **3**:397–408.
50. **Shultz, L. D., P. A. Schweitzer, T. V. Rajan, T. Yi, J. N. Ihle, R. J. Matthews, M. L. Thomas, and D. R. Beier.** 1993. Mutations at the murine motheaten locus are within the hematopoietic cell protein tyrosine phosphatase (Hcph) gene. Cell, **73**:1445–1454.
51. **Silvennoinen, O., B. Witthuhn, F. W. Quelle, J. L. Cleveland, T. Yi, and J. N. Ihle.** 1993. Structure of the JAK2 protein tyrosine kinase and its role in IL-3 signal transduction. Proc. Natl. Acad. Sci. USA, **90**:8429–8433.
52. **Sorensen, P., A. L. F. Mui, and G. Krystal.** 1989. Interleukin-3 stimulates the tyrosine phosphorylation of the 140-kilodalton interleukin-3 receptor. J. Biol. Chem. **264**:19253–19258.
53. **Sorensen, P. H., A. L. Mui, S. C. Murthy, and G. Krystal.** 1989. Interleukin-3, GM-CSF, and TPA induce distinct phosphorylation events in an interleukin 3-dependent multipotential cell line. Blood **73**:406–418.
54. **Spivak, J. L., J. Fisher, M. A. Isaacs, and W. D. Hankins.** 1992. Protein tyrosine kinases and phosphatases are involved in erythropoietin-mediated signal transduction. Exp. Hematol. **20**:500.
55. **Takaki, S., S. Mita, T. Kitamura, S. Yonehara, N. Yamaguchi, A. Tominaga, A. Miyajima, and K. Takatsu.** 1991. Identification of the second subunit of the murine interleukin-5 receptor: interleukin-3 receptor-like protein, AIC2B is a component of the high affinity interleukin-5 receptor. EMBO J. **10**:2833–2838.
56. **Tohda, S., N. Nara, Y. Imai, and N. Aoki.** 1991. Effect of protein kinase inhibitors on the proliferation of leukemic cells stimulated by granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor or interleukin-3. Leukemia **5**:813–814.
57. **Tojo, A., M. Kasuga, A. Urabe, and F. Takaku.** 1987. Vanadate can replace interleukin 3 for transient growth of factor-dependent cells. Exp. Cell Res. **171**:16–23.
58. **Torigoe, T., R. O'Connor, D. Santoli, and J. C. Reed.** 1992. Interleukin-3 regulates the activity of the LYN protein-tyrosine kinase in myeloid-committed leukemic cell lines. Blood **80**:617–624.
59. **Tsui, H. W., K. A. Siminovitch, L. de Souza, and F. W. L. Tsui.** 1993. Motheaten and viable motheaten mice have mutations in the haematopoietic cell phosphatase gene. Nat. Genet. **4**:124–129.
60. **Van Zant, G., and L. Shultz.** 1989. Hematopoietic abnormalities of the immunodeficient mouse mutant, viable motheaten (*mev*). Exp. Hematol. **17**:81–87.
61. **Velazquez, L., M. Fellous, G. R. Stark, and S. Pellegrini.** 1992. A protein tyrosine kinase in the interferon alpha/beta signaling pathway. Cell **70**:313–322.
62. **Vogel, W., R. Lammers, J. Huang, and A. Ullrich.** 1993. Activation of a phosphotyrosine phosphatase by tyrosine phosphorylation. Science **259**:1611–1614.
63. **Waksman, G., D. Kominos, D. R. Robertson, N. Pant, D.**

Baltimore, R. B. Birge, D. Cowburn, H. Hanafusa, B. J. Mayer, M. Overduin, M. D. Resh, C. B. Rios, L. Silverman, and J. Kuriyan. 1992. Crystal structure of the phosphotyrosine recognition domain SH2 of v-src complexed with tyrosine-phosphorylated peptides. Nature (London) **358:**646–653.

64. **Waksman, G., S. E. Shoelson, N. Pant, D. Cowburn, and J. Kuriyan.** 1993. Binding of a high affinity phosphotyrosyl peptide to the src SH2 domain: crystal structures of the complexed and peptide-free forms. Cell **72:**779–790.
65. **Watling, D., D. Guschin, M. Muller, B. A. Witthuhn, O. Silvennoinen, J. N. Ihle, G. R. Stark, and I. M. Kerr.** Complementation of a mutant cell line defective in the interferon-τ signal transduction pathway by the protein tyrosine kinase Jak2. Submitted for publication.
66. **Witthuhn, B., F. W. Quelle, O. Silvennoinen, T. Yi, B. Tang, O. Miura, and J. N. Ihle.** 1993. JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following EPO stimulation. Cell **74:**227–236.
67. **Yeung, Y., K. L. Berg, F. J. Pixley, R. H. Angeletti, and E. R. Stanley.** 1992. Protein tyrosine phosphatase-1C is rapidly phosphorylated in tyrosine in macrophages in response to colony stimulating factor-1. J. Biol. Chem. **267:**23447–23450.
68. **Yi, T., J. L. Cleveland, and J. N. Ihle.** 1991. Identification of novel protein tyrosine phosphatases of hematopoietic cells by PCR amplification. Blood **78:**2222–2228.
69. **Yi, T., J. L. Cleveland, and J. N. Ihle.** 1992. Protein tyrosine phosphatase containing SH2 domains: characterization, preferential expression in hematopoietic cells, and localization to human chromosome 12p12-p13. Mol. Cell. Biol. **12:**836–846.
70. **Yi, T., and J. N. Ihle.** 1993. Association of hematopoietic cell phosphatase with c-Kit after stimulation with c-Kit ligand. Mol. Cell. Biol. **13:**3350–3358.
71. **Yi, T., and J. N. Ihle.** Unpublished data.
72. **Yi, T., F. W. Quelle, and J. N. Ihle.** Unpublished data.