Characterization of Insulin-like Growth Factor I (IGF-I) Receptor Mutants for Their Effects on IGF-I- and Interleukin 4-mediated DNA Synthesis of 32D Cells*

Received for publication, March 15, 2001, and in revised form, April 18, 2001
Published, JBC Papers in Press, April 25, 2001, DOI 10.1074/jbc.M102358200

Alan Yam‡, Teresa Hyun§, and Weiqun Li‡‡

From the ¶Laboratory of Cellular and Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland 20892

Recently we demonstrated that overexpression of the wild type insulin-like growth factor I receptor (IGF-IR WT) in 32D myeloid progenitor cells led to cell proliferation in response to interleukin 4 (IL-4) as well as insulin-like growth factor I (IGF-I) in the absence of insulin receptor substrate expression (Soon, L., Flechner, L., Gutkind, J. S., Wang, L. H., Baserga, R., Pierce, J. H., and Li, W. (1998) Mol. Cell. Biol. 18, 6396–6398). To understand the structural importance of insulin-like growth factor I receptor (IGF-IR) in mediating IL-4- and IGF-I-induced DNA synthesis, we transfected various mutants of IGF-IR to 32D cells. Our results show that most mutants, including Y1250F, Y1251F, Y1250F/Y1251F, S1280A/S1281A/S1282A/S1283A, Y1316F, and Y1245d, still retained mitogenic response toward IGF-I or IL-4. However, the Y950F, Y1131F, and Y1135F mutants were not able to respond to either ligand. The H1293F/K1294R and 1293d mutants reduced response toward IGF-I but not to IL-4. Phosphorylation of Shc was greatly reduced in those three mutants that lost mitogenic response. The MAPK activity was much lower in Y1131F and Y1135F mutants, indicating the importance of the Shc/MAPK pathway in IGF-I-induced mitogenesis. Importantly, the synergistic effect of these two factors on DNA synthesis was not affected in cells expressing most of the mutants, even in those three that had lower mitogenic response toward a single ligand. These results suggest that an unidentified pathway(s) may be induced upon co-addition of IGF-I and IL-4 that sustains the intact mitogenesis.

The insulin-like growth factor I receptor (IGF-I R) belongs to the type II tyrosine kinase receptor family, sharing homology to the insulin receptor (2, 3). Both contain two α subunits responsible for ligand binding and two β subunits possessing intracellular tyrosine kinase domains. Upon ligand binding, α subunits heterodimerize with β subunits, forming a tetrameric complex leading to receptor activation, autophosphorylation, and subsequent transphosphorylation of intracellular substrates (4). Phosphorylation of two such downstream elements, Shc and insulin receptor substrate (IRS), leads to activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase pathways, respectively. Although activation of the insulin receptor is generally implicated in glucose metabolism, stimulation of the IGF-IR pathway is strongly associated with cell proliferation, malignant transformation, and anti-apoptotic effects (4–6).

Activation of IGF-IR can transmit proliferation signals in both fibroblasts and hematopoietic cell lineages (1, 7, 8). By using site-directed mutagenesis, several groups have mapped important tyrosine residues and domains of the receptor β chain responsible for various biological functions including mitogenesis, transformation, and antiapoptosis, which appeared to be nonoverlapping. Although antiapoptotic and transforming effects are somewhat interrelated and mainly dependent upon the domains located at the C terminus of the β receptor, tyrosines located at the N terminus and kinase domain are critical for mitogenic signals (9). Recently our group demonstrated that overexpression of IGF-IR allowed the 32D myeloid progenitor line to be mitogenic and proliferative in response to IGF-I treatment in the absence of IRS expression. More interestingly, interleukin 4 (IL-4), a cytokine mainly involved in lymphocyte proliferation (10), was able to induce strong mitogenesis of 32D cells overexpressing IGF-IR (32D/IGF-IR). Furthermore, synergistic effects of IGF-I and IL-4 were observed in several hematopoietic cell lines, and this synergy reached a maximum when IGF-I and IL-4 were added together to the 32D/IGF-IR line (1). These results clearly suggest that IGF-I can be a potential growth factor for hematopoietic cells. Furthermore, IL-4 may cooperate with IGF-I for hematopoietic cell proliferation.

The advantage of using the 32D cell system is that it lacks any IRS member expression. Therefore, any mitogenic signal induced upon growth factor receptor activation must utilize other pathways independent of IRS/phosphatidylinositol 3-kinase activation. To understand the structural importance of IGF-IR in transmitting mitogenic signals in response to IGF-I and IL-4, we have expressed different tyrosine to phenylalanine mutants and truncation mutants of IGF-IR in 32D cells and tested for their abilities to induce DNA synthesis. Our results show that those mutants defective for mitogenic response toward either IGF-I or IL-4 basically had reduced Shc/MAPK activation. More interestingly, all the mutants, including those defective toward one ligand and except for the ATP

---

* This work was supported in part by a grant from the Concern Foundation (to W. L.). The flow cytometric analysis was provided by the Core Facility of the Lombardi Cancer Center supported by United States Public Service Grant ZF90-CA-S1008. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ A recipient of the new investigator award from the Leukemia Research Foundation. To whom correspondence should be addressed: Dept. of Oncology, Lombardi Cancer Center, Georgetown University Medical Center, New Research Bldg., E407, 3970 Rockville Rd. NW, Washington, D. C. 20007, Tel.: 202-687-8387; Fax: 202-687-7505; E-mail: ww@georgetown.edu.

† The abbreviations used are: IGF-IR, insulin-like growth factor I receptor; IRS, insulin receptor substrate; MAPK, mitogen-activated protein kinase; IL, interleukin; STAT6, signal transducer at transcription 6; IGF-I, insulin-like growth factor I; WT, wild type; 1245d, C-terminal truncation mutant; 1293d, C-terminal truncation mutant.

This paper is available on line at http://www.jbc.org
This is an Open Access article under the CC BY license.
binding site mutation (K1003R), were fully mitogenic when both ligands were added together. Because both Shc/MAPK and signal transducer at transcription 6 (STAT6) pathways were not further enhanced by co-adding the two factors when compared with those induced by a single ligand, these results suggest that some unidentified pathways are induced to compensate for the reduced mitogenesis conferred by single ligand stimulation.

EXPERIMENTAL PROCEDURES

Establishment of IGF-IR Mutants, Cell Transfection, and Culture—Establishment of all the IGF-IR mutants has been reported previously (7, 9, 11–14). The 32D cells were transfected by electroporation as reported by Li et al. (15). Drug-selected lines were cultured with RPMI 1640 medium containing 15% fetal calf serum and 5% of the supernatant of the WEHI-3 cell line as the source of IL-3.

Mitogenic Assay—Transfectants of 32D cells were washed twice with Dulbecco’s phosphate-buffered saline. The number of cells was determined using a cell counter (Coulter). 2 × 10^5 cells were plated onto each well of 24-well plates in RPMI 1640 medium containing only 15% fetal calf serum without IL-3. Human IGF-I (Intergen) and murine IL-4 (Intergen) in the concentration of 100 ng/ml were added to each well. After 48 h in culture, the cells were pulsed with 1 μCi of [3H]thymidine (Amersham Pharmacia Biotech) for another 4 h and harvested using a cell harvester (Skatron Instruments). Dried filters were soaked in scintillation liquid, and the counts/min were measured using a β counter (Beckman). The mean values from triplicate wells were calculated together with standard deviations.

Flow Cytometric Analysis—Cells were incubated with anti-IGF-IR α chain monoclonal antibody (Oncogene Science, Inc.) for 30 min at 4 °C. Washed cells were incubated with phycoerythrin-conjugated anti-mouse IgG (CALTAG Laboratories). The cells were subjected to flow cytometry using a Becton-Dickinson FACScan.

Immunoprecipitation and Immunoblot Analysis—32D cells and transfectants were serum- and IL-3-starved for 2 h, stimulated with IGF-I (100 ng/ml) and/or IL-4 (100 ng/ml) for 10 min, and lysed in a buffer containing Triton X-100 (1%). Protein concentrations were determined by using a kit from Bio-Rad. Equivalent amounts of cell lysates were immunoprecipitated with 25 μl of anti-phosphotyrosine conjugated to protein A beads (Upstate Biotechnology, Inc.) or anti-STAT6 (1 μg/sample, Santa Cruz Biotechnologies, Inc.) together with 40 μl of protein G beads (Amersham Pharmacia Biotech). Washed immunoprecipitates were separated on 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the proteins transferred onto Immobilon membranes (Millipore) were immunoblotted with anti-Shc (Transduction Laboratories; 1:1000) or anti-phosphotyrosine (1 μg/ml, Upstate Biotechnology, Inc.). The protein bands were subsequently detected on the ECL Western blot detection system (Amersham Pharmacia Biotech). For direct immunoblot analysis, denatured protein samples (100 μg/sample) were directly subjected to SDS-polyacrylamide gel electrophoresis, and transferred proteins were immunoblotted with anti-IGF-IR β chain (1:500, Santa Cruz Biotechnologies, Inc.).

MAPK Activity Assay—Detailed description of the method has been reported previously (1, 17).

RESULTS

Expression of the Various Mutants of IGF-IR in 32D Cells—To understand the structural and functional relation of IGF-IR in IGF-I- and IL-4-mediated mitogenesis, we overexpressed the various tyrosine to phenylalanine mutants and truncation mutants of the β chain of IGF-IR in 32D myeloid cells. As shown in Fig. 1A, transfection of wild type IGF-IR (IGF-IRWT) and many other mutants, including Y950F, Y1131F, Y1135F, Y1250F, Y1251F, Y1250F/Y1251F, S1280A/S1281A/S1282A/S1283A, H1293F/K1294R, and Y1316F, resulted in 3–10-fold increases in protein expression levels when compared with endogenous IGF-IR as determined by anti-IGF-IR β chain immunoblot analysis (endogenous IGF-IR level was detectable after longer exposure (1)). We were not able to detect the expression of two C-terminal truncation mutants (1245Δ and 1293Δ) by direct Western blotting using the anti-IGF-IR β chain antibody because these truncation mutants lost the C-terminal portion of the β chain (18) where the peptide antibody was generated. However, when a monoclonal antibody against the extracellular domain of the α chain was used in a flow cytometric analysis, expression of these truncation mutants was easily detectable (Fig. 1B).

Mitogenic Responses of the Various IGF-IR Mutants Expressed in 32D Cells in Response to IGF-I and/or IL-4—The 32D cells and the various transfectants were subjected to a mitogenic assay in the presence of IGF-I, IL-4, or the two factors together. As shown in Fig. 2, IL-4 treatment of the parental line resulted in some increases in DNA synthesis. This IL-4-mediated mitogenesis was enhanced upon the expression of IGF-IRWT as seen in both WT-1 (low IGF-IR expression) and WT-2 (high IGF-IR expression) lines. Overexpression of IGF-IR in WT-1 and WT-2 lines allowed for great induction of mitogenesis in response to both IGF-I and IL-4, consistent with the previous report (1). Co-addition of IGF-I and IL-4 resulted in the maximal mitogenesis in both WT-1 and WT-2 clones, confirming the synergistic effect of these two factors. Expression of an ATP binding mutant of IGF-IR (K1003R) completely abolished mitogenesis in response to both growth factors, either alone or together, emphasizing the role of IGF-IR activity, which synergizes with IL-4 for DNA synthesis.

Tyrosine 950 of the IGF-IR β chain is known to be the site of interaction with the phosphotyrosine binding domains of Sbc and IRS upon its phosphorylation (19–22). Mutation of this site to a phenylalanine abolishes IGF-I-mediated mitogenic and
functions of IGF-I Receptor in 32D Cell Proliferation

**Fig. 2.** DNA synthesis of the various mutants of IGF-IR expressed in 32D cells in response to IGF-I and/or IL-4 stimulation. The 32D cells and various transfectants were washed twice with Dulbecco's phosphate-buffered saline and maintained in RPMI 1640 medium containing 15% fetal calf serum in the presence of the various ligands at the concentration of 100 ng/ml each. [³H]Thymidine was added after a period of 44 h in culture. Cells were harvested, and the number of counts/min (cpm) was measured. The first bar of each cell line represents counts/min of cells without stimulation, whereas the second, third, and fourth bars represent counts/min of IL-4 (100 ng/ml), IGF-I (100 ng/ml), and IL-4 and IGF-I (100 ng/ml each) of each stimulation, respectively, in each cell line. Error bars indicate standard deviations. Asterisks indicate statistical significance of reduced DNA synthesis of the mutants (p < 0.01) when compared with that induced by same ligand(s) of WT-2 in the same assay. KR, K1003R; 950F, Y950F; 1131F, Y1131F; 1135F, Y1135F, 1250F, Y1250F; 1251F, Y1251F; 50F/51F, Y50F/Y51F, Y1250F/Y1251F; S4A, S1280A/S1281A/S1282A/S1283A; 93F/94F, H1293F/K1294R; 1136F, Y1136F.

transforming activities in fibroblasts (13, 23), but its antiapoptotic effect is retained (9). Here we have shown that the Y950F mutant lost its capability of inducing mitogenesis in response to IGF-I or IL-4. Similarly, the tyrosine to phenylalanine mutants at the kinase domain (Y1131F and Y1135F), originally shown to affect the kinase activity (8, 24), were unable to transmit mitogenic signals to either IGF-I or IL-4.

Most tyrosine residues located at the C terminus of the kinase domain did not affect the mitogenesis in response to either IGF-I or IL-4 stimulation, including the Y1250F, Y1251F, Y1250F/Y1251F, and Y1316F mutants. Mutation on these sites did not affect the mitogenesis of fibroblasts either (13, 23). The S1280A/S1281A/S1282A/S1283A mutant has been defined to be important for cell transformation but was capable of inducing mitogenesis in both fibroblasts (12) and myeloid cells as shown in Fig. 2. The H1293F/K1294R mutant replaced the two important basic residues (histamine and lysine) within an 8-amino acid stretch, which is not shared by the insulin chain, we analyzed tyrosine phosphorylation of STAT6 in response to IL-4 and/or IGF-I. As shown in Fig. 3, tyrosine phosphorylation of STAT6 was increased in response to IL-4 stimulation in the two WT transfectants. Consistent with the previous report (1), expression of the K1003R mutant suppressed STAT6 phosphorylation (1), suggesting that STAT6 may be involved in IL-4-induced mitogenesis upon IGF-IR overexpression. To investigate the role of STAT6 in mitogenesis upon mutating different sites of the β chain, we analyzed tyrosine phosphorylation of STAT6 in response to IL-4 and/or IGF-I. STAT6 Activation Does Not Generally Correlate with Mitogenic Response in Individual Mutants—STAT6 has been known to be an important downstream molecule of IL-4 signaling (10, 25, 26). Its phosphorylation was increased in the 32D/IGF-IRWT line in response to IL-4 (1) (Fig. 3). In contrast, expression of the K1003R mutant suppressed STAT6 phosphorylation (1), suggesting that STAT6 may be involved in IL-4-induced mitogenesis upon IGF-IR overexpression. To investigate the role of STAT6 in mitogenesis upon mutating different sites of the β chain, we analyzed tyrosine phosphorylation of STAT6 in response to IL-4 and/or IGF-I. As shown in Fig. 3, tyrosine phosphorylation of STAT6 was increased in response to IL-4 stimulation in the two WT transfectants. Consistent with the previous report (1), expression of the K1003R mutant suppressed STAT6 phosphorylation. Phosphorylation induced by IL-4 was higher in Y950F and Y1136F but lower in Y1131F, Y1135F, Y1250F, Y1251F, Y1250F/Y1251F, and S1280A/S1281A/S1282A/S1283A transfectants when compared with 32D cells. No significant increase in its phosphorylation was observed in any cell lines treated with IL-4 plus IGF-I in comparison with IL-4 alone, suggesting that the synergistic effect imposed by IL-4 and IGF-I may not be directly linked to STAT6 activation. Although we do not believe that STAT6 may affect the mitogenic response of each individual mutant directly, no definite conclusion can be drawn to exclude its involvement in WT receptor-mediated mitogenesis in response to IL-4 because we reproducibly observed the increase in its phos-
Function of IGF-I Receptor in 32D Cell Proliferation

|  | 32D | WT-1 | KR | 950F | 1131F |
|---|---|---|---|---|---|
| IGF-I | - | + | - | + | - |
| IL-4 | - | + | - | - | - |

|  | 32D | WT-1 | 950F | 1131F |
|---|---|---|---|---|
| IGF-I | - | - | - | - |
| IL-4 | - | - | - | - |

**Fig. 4. Tyrosine phosphorylation of Shc is reduced in Y950F, Y1131F, and Y1135F mutants of IGF-IR in response to IGF-I stimulation.** 32D cells and the various transfectants were serum- and IL-3-starved for 2 h, either untreated or stimulated with IGF-I, IL-4, or both together for 10 min, and lysed. Equivalent amounts of cell lysates were immunoprecipitated with anti-phosphotyrosine antibody. Tyrosine-phosphorylated p52 Shc is shown. KR, K1003R; 950F, Y950F; 1131F, Y1131F; 1135F, Y1135F; 1250F, Y1250F; 1251F, Y1251F; 1250F/1251F, Y1250F/Y1251F; S4A, S1280A/S1281A/S1282A/S1283A; 1293F/1294R, H1293F/K1294R; 1316F, Y1316F.

**Fig. 5. MAPK activity assay** using myelin basic protein as the substrate. Phosphorylated myelin basic protein is shown after autoradiography. Y950F, Y950F; 1131F, Y1131F; 1135F, Y1135F; 1250F, Y1250F; 1293F/1294R, H1293F/K1294R; KR, K1003R.

**MAPK Activity Assay**

**DISCUSSION**

In the present study, we have attempted to dissect the roles of different tyrosine residues and domains within the IGF-IR β chain in both IGF-I- and IL-4-mediated DNA synthesis upon receptor overexpression. We took advantage of using 32D myeloid progenitor cells because these cells did not elicit a significant mitogenic response to either IGF-I or IL-4 without IGF-IR overexpression. However, expression of the IGF-IR rendered this cell line fully mitogenic, not only to IGF-I but also to IL-4. Our results showed that three mutants, including Y950F, Y1131F, and Y1135F, lost response to both IGF-I and IL-4. Two other mutants involving the basic residue stretch (H1293F/K1294R and 1293d) partially reduced their mitogenic response to IGF-I but not to IL-4. Finally, we showed that co-addition of IL-4 and IGF-I can compensate for the reduced DNA synthesis that occurred with stimulation with one ligand in several mutant transfectants, such as Y950F, Y1131F, Y1135F, H1293F/K1294R, and 1293d mutants, through some pathways obviously not affected by expression of these mutants.

Tyrosine 950 of the IGF-IR or Tyr-960 of the insulin receptor has been known to bind to IRS and Shc upon its phosphorylation (19–22). Accordingly, its mutation resulted in less phosphorylation of IRS-1 and diminished DNA synthesis, cell growth, and transforming activity in fibroblasts (14). Very interestingly, mutation on this site did not affect the ability of the IGF-IR to protect hematopoietic cells and fibroblasts from death, a process involving MAPK downstream of Shc activation, in addition to MAPK, may be involved in mitogenesis induced upon IGF-IR overexpression and activation. The 1293d and H1293F/K1294R mutants still possessed higher levels of MAPK activity than that of 32D cells, supporting our hypothesis that some other pathways not involving MAPK downstream of Shc may play a role in transmitting mitogenic signal in response to IGF-I.

**Shc/MAPK Pathway Activation Is Critical for IGF-IR-mediated Mitogenesis**—We previously showed that the Shc/MAPK pathway was greatly induced in response to IGF-I upon IGF-IR overexpression (1). Furthermore, specific inhibitors of MAPK kinase suppressed mitogenesis induced by either IGF-I or IL-4 (1). To seek further insight into the structural importance of IGF-IR in transmitting a mitogenic signal through the Shc/MAPK pathway, we first tested for Shc phosphorylation. As shown in Fig. 4, tyrosine phosphorylation of p52 Shc was greatly increased in the WT transfectants when compared with that of 32D cells in response to IGF-I stimulation. Again, expression of the K1003R mutant completely abolished Shc phosphorylation. Correlating with the reduced mitogenesis toward IGF-I (Fig. 2), the Y950F, Y1131F, and Y1135F mutants had reduced Shc phosphorylation. In contrast, the other mutants did not show any reduction in Shc phosphorylation. Because Shc phosphorylation was not further enhanced by IL-4 and IGF-I, it seems unlikely that Shc phosphorylation and subsequent MAPK activation (see Fig. 5) play a major role in the synergistic effect.

Subsequent MAPK assay using the myelin basic protein as a substrate clearly showed that MAPK activities were greatly induced upon overexpression of the WT receptor (Fig. 5). Again, its activation was fully suppressed by K1003R expression. Although expression of the kinase domain tyrosine mutants (Y1131F and Y1135F) possessed reduced MAPK activity, correlating with the reduced kinase activity (8, 11, 24) and Shc phosphorylation (Fig. 4), the MAPK activity detected in the Y950F mutant was similar to that of the WT line in response to IGF-I. This result suggests that Shc phosphorylation may be more important than MAPK activity in determining mitogenesis in response to IGF-I. Alternatively, some other pathways downstream of Shc activation, in addition to MAPK, may be involved in mitogenesis induced upon IGF-IR overexpression and activation. The 1293d and H1293F/K1294R mutants still possessed higher levels of MAPK activity than that of 32D cells, supporting our hypothesis that some other pathways not involving MAPK downstream of Shc may play a role in transmitting mitogenic signal in response to IGF-I.
apoptosis induced by cytokine withdrawal and myc gene activation, respectively (9). These results clearly indicate that some other pathways, independent of Shc and IRS activation, determine the effect of IGF-IR on suppressing apoptosis. Our results not only confirm the previous data indicating that phosphorylation on this site is critical for cell proliferation in hematopoietic cells toward IGF-I but also support the role of 950 phosphorylation in mediating IL-4-induced mitogenic response. Because 32D cells do not express any members of the IRS family, the effect of this mutation on mitogenesis must reside in Shc or some other unidentified pathways linked to tyrosine 950 phosphorylation. Biochemically, we have found that loss of phosphorylation on this site significantly affected Shc phosphorylation, thus confirming data from the two-hybrid yeast system, in which Shc interaction with the insulin receptor was defined through this site (19–22). One very interesting phenomenon was that MAPK activation was not affected by this mutation despite Shc phosphorylation being greatly reduced, suggesting that the Shc phosphorylation does not fully correlate with MAPK activation. This result could also mean that MAPK activation may not be the only pathway driving 32D cell proliferation in response to IGF-IR overexpression and activation.

Tyrosines 1131 and 1135, forming the tyrosine cluster with tyrosine 1136 and located in the kinase domain, are the major autophosphorylation sites in response to IGF-I stimulation (8, 11, 24). Previous results using single mutants indicated that tyrosines 1131 and 1135 were not very important for monolayer cell growth of fibroblasts in response to IGF-I (11, 27). On the other hand, a single mutation on tyrosine 1136 significantly impaired this function (11). Combinations of mutations of two of the three tyrosines or the mutation of all three sites abolished IGF-IR-mediated short term (autophosphorylation, IRS-1 and Shc phosphorylation, and IRS-1 and Shc interaction with Grb2) and long term functions (cell proliferation and tumorigenicity) (8). The triple mutant also abolished oncogenic IGF-IR-mediated transformation in both chicken and mouse fibroblasts (23). On the other hand, mutations in the cluster did not affect the antiapoptotic effect of IGF-IR, again arguing for the distinctive pathways responsible for different biological functions (9). Our results using these two single mutants clearly indicate that they are very important for Shc/MAPK activation and for cell proliferation. This is different from the results of expressing single mutants in fibroblasts and may reflect the more negative nature of using hematopoietic cells in the mitogenic assays. Although we were not able to express the triple mutant (Y1131F/Y1135F/Y1136F) in our system, we speculate it would also affect the DNA synthesis toward IGF-I and/or IL-4 because this mutation is defective for the tyrosine kinase activity.

STAT6 is known to be critical for IL-4-induced gene expression and biological functions (10, 25, 26). We previously showed that STAT6 phosphorylation in response to IL-4 correlated with the IGF-IR activity, suggesting that enhanced STAT6 pathway may be involved in IL-4-induced mitogenesis upon IGF-IR overexpression (1). However, STAT6 phosphorylation was not always in accordance with the mitogenic abilities of each mutant analyzed in this study. Although this result may exclude the importance of STAT6 activation in Y950F-, Y1250F-, Y1251F- and S1280A/S1281A/S1282A/S1283A-mediated DNA synthesis in response to IL-4, whether STAT6 is still necessary, but not sufficient, for WT and Y1131F and Y1135F mutants to cooperate with the IL-4 pathway for cell proliferation remains to be tested. Likewise, the pathways involved in IL-4-mediated mitogenesis of 32D cells overexpressing IGF-IR warrant further investigation.

Of great interest in this study is the effect of mutants on the synergistic mitogenesis toward IGF-I and IL-4. Except for the abolished mitogenic response demonstrated by expressing the K1003R mutant, all other mutants analyzed were able to mediate DNA synthesis in the presence of these two factors. Although these data emphasize the role of basal activity of the IGF-IR in the synergy, the corresponding pathway(s) responding to these two factors has not been delineated. Because co-addition of the factors did not affect IL-4-induced STAT6 phosphorylation (Fig. 3) or IGF-I-induced Shc phosphorylation (Fig. 4) and MAPK activation (data not shown), some other novel pathways may be induced in the presence of these two growth factors. Our previous results showed that c-myc gene induction correlated well with the synergy observed in both the parental 32D line and the 32D/IGF-IR transfectant (1), suggesting that early response genes may be induced for the biologic effect. We are currently attempting to utilize the microarray technique to search for more genes involved in this synergistic effect. Studies along this line may allow us to design new methods of treating hematopoietic malignancies in which IGF-I- and IL-4-initiated pathways are abnormally activated.

Acknowledgment—We thank Renato Baserga for all the IGF-IR mutant constructs and for helpful discussion.

REFERENCES

1. Soon, L., Flechner, L., Gutkind, J. S., Wang, L. H., Baserga, R., Pierce, J. H., and Li, W. (1999) Mol. Cell. Biol. 19, 3816–3828
2. Ulrich, A., and Schlessinger, J. (1990) Cell 61, 203–211
3. Ulrich, A., Gray, A., Tam, R. W., Yang-Feng, T., Tsubokawa, M., Collins, C., Henzel, W., Le Bon, T., Kathuria, S., Chen, E., Jacobs, S., Frank, U., Ramachandran, J., and Fujita-Yamaguchi, Y. (1986) EMBO J. 5, 2503–2512
4. Baserga, R., Hongo, A., Rubini, M., Prisco, M., and Valentini, B. (1997) Biochim. Biophys. Acta 1322, F105–F126
5. Blakesley, V. A., Scrimgeour, A., Esposito, D., and LeRoith, D. (1996) Cytochrome Growth Factor Rev. 7, 153–159
6. Baserga, R. (1985) Cancer Res. 45, 249–252
7. Valentini, R., Romano, G., Peruzzi, F., Morrione, A., Prisco, M., Soddu, S., Cristofanelli, B., Sacchi, A., and Baserga, R. (1999) J. Biol. Chem. 274, 12425–12430
8. Hernandez-Sanchez, C., Blakesley, V., Kalebic, T., Helman, L., and LeRoith, D. (1995) J. Biol. Chem. 270, 29176–29181
9. O’Connor, R., Kaufmann-Zeh, A., Liu, Y., Lehara, S., Evan, G. I., Baserga, R., and Blatter, W. A. (1997) Mol. Cell. Biol. 17, 427–435
10. Keegan, A. D., Neils, K., Wein, L. M., Pierce, J. H., and Paul, E. W. (1994) Immunol. Today 15, 423–432
11. Li, S., Ferber, A., Miura, M., and Baserga, R. (1994) J. Biol. Chem. 269, 32558–32564
12. Li, S., Resnicoff, M., and Baserga, R. (1996) J. Biol. Chem. 271, 12254–12260
13. Miura, M., Surnace, E., Burgaud, J.-L., and Baserga, R. (1995) J. Biol. Chem. 270, 22639–22644
14. Miura, M., Li, S., and Baserga, R. (1995) Cancer Res. 55, 663–667
15. Li, W., Hyun, T., Heller, M., Yam, A., Flechner, L., Pierce, J. H., and Rudikoff, S. (2000) Cancer Res. 60, 3909–3915
16. Li, W., Jiang, Y.-C., Zhang, J., Soon, L., Flechner, L., Kapoor, V., Pierce, J. H., and Wang, L.-H. (1998) Mol. Cell. Biol. 18, 5888–5898
17. Alimandi, M., Heidaran, M. A., Gutkind, J. S., Zhang, J., Ellmore, N., Valius, S., Kazlauskas, A., Pierce, J. H., and Li, W. (1997) Oncogene 15, 585–593
18. Hongo, A., D’Ambrosio, C., Miura, M., Morrione, A., and Baserga, R. (1996) Oncogene 12, 1231–1238
19. Gustafson, T. A., He, W., Craparo, A., Schaub, C. D., and O’Neill, T. J. (1995) Mol. Cell. Biol. 15, 2500–2508
20. He, W., O’Neill, T. J., and Gustafson, T. A. (1995) J. Biol. Chem. 270, 23258–23262
21. He, W., Craparo, A., Zhu, Y., O’Neill, T. J., Wang, L. M., Pierce, J. H., and Gustafson, T. A. (1996) J. Biol. Chem. 271, 11641–11645
22. O’Neill, T. J., Craparo, A., and Gustafson, T. A. (1994) Mol. Cell. Biol. 14, 6433–6442
23. Jiang, Y., Chan, J.-L.-K., Zong, C. S., and Wang, L.-H. (1996) J. Biol. Chem. 271, 160–167
24. Kato, H., Faria, T. N., Stannard, B., Roberts, C. T., and LeRoith, D. (1994) Mol. Endocrinol. 8, 50–59
25. Ibe, J. N., Nosaka, T., Thierfelder, W., Quelle, F. W., and Shimoda, K. (1997) Stem Cells 15, 105–111
26. Ibe, J. N., Stravopodis, D., Parganas, E., Thierfelder, W., Feng, J., Wang, D., and Teguld, S. (1998) Cancer J. Sci. Am. 4, 584–591
27. Stannard, B., Blakesley, V., Kate, H., Roberts, C. T., Jr., and LeRoith, D. (1995) Endocrinology 136, 4918–4924