Immunoreceptor Tyrosine-Based Inhibitory Motif of the IL-4 Receptor Associates with SH2-Containing Phosphatases and Regulates IL-4-Induced Proliferation

Masaki Kashiwada, Cosmas C. Giallourakis, Ping-Ying Pan and Paul B. Rothman

*J Immunol* 2001; 167:6382-6387; doi: 10.4049/jimmunol.167.11.6382

http://www.jimmunol.org/content/167/11/6382

References

This article cites 49 articles, 24 of which you can access for free at:
[http://www.jimmunol.org/content/167/11/6382.full#ref-list-1](http://www.jimmunol.org/content/167/11/6382.full#ref-list-1)

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
[http://jimmunol.org/subscription](http://jimmunol.org/subscription)

Permissions

Submit copyright permission requests at:
[http://www.aai.org/About/Publications/JI/copyright.html](http://www.aai.org/About/Publications/JI/copyright.html)

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
[http://jimmunol.org/alerts](http://jimmunol.org/alerts)
Immuneceptor Tyrosine-Based Inhibitory Motif of the IL-4 Receptor Associates with SH2-Containing Phosphatases and Regulates IL-4-Induced Proliferation

Masaki Kashiwada, Cosmas C. Giallourakis, Ping-Ying Pan, and Paul B. Rothman

Immuneceptor tyrosine-based inhibitory motifs (ITIM) have been implicated in the negative modulation of immuneceptor signaling pathways. The IL-4R α-chain (IL-4Rα) contains a putative ITIM in the carboxyl terminal. To determine the role of ITIM in the IL-4 signaling pathway, we ablated the ITIM of IL-4Rα by deletion and site-directed mutagenesis and stably expressed the wild-type (WT) and mutant hIL-4Rα in 32D/insulin receptor substrate-2 (IRS-2) cells. Strikingly, 32D/IRS-2 cells expressing mutant human (h)IL-4Rα were hyperproliferative in response to IL-4 compared with cells expressing WT hIL-4Rα. Enhanced tyrosine phosphorylation of Stat6, but not IRS-2, induced by hIL-4 was observed in cells expressing mutant Y713F. Using peptides corresponding to the ITIM of hIL-4Rα, we demonstrate that tyrosine-phosphorylated peptides, but not their nonphosphorylated counterparts, coprecipitate SH2-containing tyrosine phosphatase-1, SH2-containing tyrosine phosphatase-2, and SH2-containing inositol 5′-phosphatase. The in vivo association of SH2-containing inositol 5′-phosphatase with IL-4Rα was verified by coinmuno precipitation with anti-IL-4R Abs. These results demonstrate a functional role for ITIM in the regulation of IL-4-induced proliferation.


Interleukin-4 is a pleiotropic cytokine that regulates growth and differentiation in various hemopoietic and nonhemopoietic cells (1). In hemopoietic cells, IL-4 exerts its multiple functions by binding to a high affinity receptor complex composed of IL-4R α-chain (IL-4Rα) and common γ-chain (2–5). Upon ligand engagement, IL-4Rα is tyrosine-phosphorylated, creating docking sites for downstream signaling molecules. Five conserved tyrosine residues that can potentially be phosphorylated are present in the cytoplasmic domain of IL-4Rα (6). Phosphorylation of tyrosine 497 (Y497) has been shown to mediate mitogenic responses through recruitment and phosphorylation of insulin receptor substrate (IRS-1) and/or IRS-2 in nonhemopoietic and hemopoietic cells, respectively (7–9). In contrast, IL-4-mediated gene activation and differentiation were mapped to a separate region of IL-4Rα (7–9). Phosphorylation of tyrosine 713 (Y713), whose function in IL-4-mediated gene activation, growth, and differentiation remains undetermined. A previous study demonstrated that mutation of Y713 alters the ability of IL-4 to prevent apoptosis (10). The amino acid sequence flanking Y713 resembles the canonical consensus (I/VxYxL) of the immuneceptor tyrosine-based inhibitory motif (ITIM) (13).

ITIM was first identified in the cytoplasmic domain of FcγRIIB and has been recognized in a growing family of inhibitory receptors (14, 15). The role of ITIM in the negative modulation of immuneceptor signaling has been demonstrated in FcγRIIB (16), killer cell inhibitory receptors (17, 18), CD22 (19), and CTLA-4 (20) signal pathways. Accumulating evidence reveals that the ITIM, when phosphorylated on tyrosine, becomes a docking site for SH2-containing tyrosine phosphatase-1 (SHP-1) and/or SH2-containing inositol 5′-phosphatase (SHP) (21–25). In addition, SHP-2 has been shown to be associated with the ITIM of FcγRIIB and CTLA-4 (21, 26–28). After recruitment to the inhibitory receptors, these phosphatases regulate signaling cascades initiating from these receptors, presumably by dephosphorylating activated signaling molecules.

Recent evidence suggests that there may be functional ITIM motifs present in the cytoplasmic domains of cytokine receptors. For example, the cytoplasmic domain of erythropoietin receptors (EPO-R) contains an ITIM that mediates its regulatory role in proliferative signals by recruiting SHP-1 (29). Our examination of the cytoplasmic domain of IL-4Rα suggests that it contains a putative ITIM. Here we report that ablation of the IL-4Rα ITIM results in a hyperproliferative response to IL-4 stimulation, which correlates with increased activation of Stat6, but not IRS-2. Biochemical studies demonstrate that SHP-1, SHP-2, and SHIP can associate with the IL-4Rα ITIM. These data suggest that the IL-4Rα contains a functional ITIM.

Abbreviations used in this paper: IRS, insulin receptor substrate; EPO-R, erythropoietin receptor; GFP, green fluorescence protein; ITIM, immuneceptor tyrosine-based inhibitory motifs; hIL-4Rα, human IL-4Rα; SHP-1, SH2-containing tyrosine phosphatase 1; WT, wild type; MSCV, murine stem cell virus; GAS, γ activation site.

Departments of Medicine and Microbiology, College of Physicians and Surgeons, Columbia University, New York, NY 10032

Received for publication October 20, 2000. Accepted for publication September 24, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked

1 This work was supported by National Institutes of Health Grant P01AI50514 and a grant from the Asthma and Allergy Foundation of America (to P.B.R.); and a Howard Hughes Medical Institute Medical Student Fellowship (to C.C.G.). P.B.R. was a Scholar of the Leukemia and Lymphoma Society.

2 Address correspondence and reprint requests to Dr. Paul Rothman, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, NY 10032. E-mail address: pbr3@columbia.edu

3 Abbreviations used in this paper: IRS, insulin receptor substrate; EPO-R, erythropoietin receptor; GFP, green fluorescence protein; ITIM, immuneceptor tyrosine-based inhibitory motifs; hIL-4Rα, human IL-4Rα; SHP-1, SH2-containing tyrosine phosphatase 1; WT, wild type; MSCV, murine stem cell virus; GAS, γ activation site.

Copyright © 2001 by The American Association of Immunologists

0022-1767/01/$02.00

Downloaded from http://www.jimmunol.org/ by guest on May 2, 2017
Materials and Methods
Ab, cytokines, and peptides
Anti-SHIP and anti-SHIP-1 Abs were gifts from Dr. K. M. Coggeshall (Ohio State University, Columbus, OH) and Dr. B. Neel (Harvard Medical School, Boston, MA), respectively. Anti-SHIP-2 and anti-Jak1 Abs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); anti-Shc, anti-IRS, anti-Stat6, anti-Jak1 Abs, anti-phosphotyrosine mAb 4G10, and HRP-conjugated anti-rabbit Ig were obtained from Upstate Biotechnology (Lake Placid, NY); anti-human (h)IL-4R mAb was purchased from R&D Systems (Minneapolis, MN); and biotinylated goat anti-mouse Ig Abs and avidin-conjugated APC were purchased from BD PhaMingen (San Diego, CA). Murine IL-4 and hIL-4 were gifts from Dr. R. Coffman (DNAx Research Institute, Palo Alto, CA) and Dr. S. Narula (Schering-Plough Corp., Kenilworth, NJ), respectively. Biotinylated synthetic nonphosphorylated or tyrosine-phosphorylated peptides corresponding to the sequence flanking the ITIM of hIL-4R were purchased from Quality Control Biologicals (Hopkinton, MA). The amino acid sequence of the peptide is SLGSGIVYSALTCHLC.

Expression plasmids
Human IL-4Rα deletion mutant (Δ712) was generated by restriction digestion of wild-type (WT) hIL-4Rα plasmids provided by Dr. William Paul (National Institutes of Health, Bethesda, MD) and Dr. J. Ryan (Virginia Commonwealth University, Richmond, VA) with AccI. The Altered Sites II in vitro mutagenesis system (Promega, Madison, WI) was used to generate the point mutation (Y713F) of the critical tyrosine residue in the ITIM of hIL-4Rα according to the manufacturer’s instructions. The resulting fragments, Δ712, Y713F, and WT hIL-4Rα cDNA, were subcloned into murine stem cell virus (MSCV)-internal ribosomal entry site promoter-green fluorescence peptide (GFP) vector (provided by Dr. G. Nolan, Stanford University, Palo Alto, CA). The hIL-4Rα- and GFP were expressed as a bicistronic message, and internal ribosomal entry site allows concomitant expression of both hIL-4Rα and GFP.

Cell culture, retrovirus production, and establishment of stable cell lines
M12 cells were cultured in complete RPMI 1640 (RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, and 50 μM Na3VO4, 50 mM NaF, 1 mM dithiothreitol, 0.4 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 3 μg/ml aprotinin, and 2 μg/ml pepstatin. M12 cells were cultured in complete RPMI 1640 for 30 min. Immunoprecipitation was resolved on 10% SDS-PAGE and transferred onto nitrocellulose membrane. Cell lysates were precipitated for 4 h at 4°C. To produce retroviral stocks carrying hIL-4Rα, we used GFP as a positive selection marker and FACS to collect cells expressing GFP and hIL-4Rα- or GFP-only expressing cells. The expression level of hIL-4Rα correlated with that of GFP.

Peptide pulldown, immunoprecipitation, and Western blot analysis
Biotinylated peptides corresponding to hIL-4Rα ITIM were incubated with streptavidin-conjugated beads (Sigma) at room temperature for 1 h, and subsequently free peptides were removed by washing three times with lysis buffer. M12 cells were stimulated with murine IL-4 for 10 min. Whole cell extracts were prepared with lysis buffer consisting of 1% Nonidet P-40, 50 mM Tris (pH 8.0), 10% glycerol, 0.1 M EDTA, 150 mM NaCl, 100 μM Na3VO4, 50 mM NaF, 1 mM dithiothreitol, 0.4 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 3 μg/ml aprotinin, and 2 μg/ml pepstatin. Cell lysates were precipitated for 4 h at 4°C with nonphosphorylated or phosphorylated peptides coupled to agarose beads. The adsorbed material was resolved on 10% SDS-PAGE and transferred onto nitrocellulose membrane. 32D/IRS-2 cells were IL-3-starved for 4 h and were either left untreated or treated with hIL-4 (100 ng/ml) for 30 min. Immunoprecipitation and Western blot analysis were performed as previously described (30).

Proliferation assay
32D/IRS-2 cells expressing hIL-4Rα- and GFP were passaged 3 days before the experiment. On the day of the experiment cells were washed three times with complete RPMI 1640 with 5% WEHI-3-conditioned medium, and then cultured at a density of 2.5 × 104/200 μl in a 96-well flat-bottom microtiter plate in culture medium with varying concentrations of recombinant hIL-4 or 5% WEHI-3-conditioned medium as indicated. Cells were pulse-labeled with 1 μCi [3H]thymidine (New England Nuclear, Boston, MA) for the last 6–8 h of the culture time and harvested at 53 h after adding IL-4. [3H]Thymidine incorporation was measured by a scintillation counter.

Electrophoretic mobility shift assay
32D/IRS-2 cells expressing WT or mutant hIL-4Rα were stimulated with 50 ng/ml hIL-4 for 0, 2, 6, 12, 24, 48, and 96 h. Whole cell extracts were prepared, and mobility shift reactions were performed as described previously (30). The probes were used from IRF-1 γ activation site (GAS) element (5'-GATTCCCCGAAAT-3' for Staf6) and from octamer consensus sequence (5'-TGGCGAATGCAATCAGAA-3' for Oct-1).

Results and Discussion
The cytoplasmic domain of IL-4Rα contains an ITIM
Tyrosine 713 (Y713) of the IL-4Rα is the only conserved tyrosine whose function remains unknown. We were interested in the biological function of Y713. Alignment of the region flanking Y713 of IL-4Rα with the known ITIM sequences from CD22, killer cell inhibitory receptors, FcγRIIB, and human EPO-R shows apparent canonical consensus of the ITIM in the human and murine IL-4Rα-α-chains (Fig. 1). Among the five conserved tyrosine residues in the cytoplasmic region of IL-4Rα-α-chain, only the sequence flanking Y713 resembles the consensus of the ITIM (IVxYxxL) (13).

Ablation of the IL-4Rα ITIM results in a hyperproliferative response to IL-4 stimulation
To assess the physiological role of the IL-4Rα ITIM, we generated 32D/IRS-2 cells expressing WT hIL-4Rα (WT825) and mutant IL-4Rα lacking the ITIM (Δ712 and Y713F). To avoid clonal variation among stable cell lines, we used GFP as a positive selection marker and FACS to collect cells expressing GFP and hIL-4Rα-α-chains, so that all stable cell lines used in this report are polyclonal in nature. We also took advantage of the fact that murine and human IL-4 do not cross-react, so that endogenous murine IL-4Rα would not interfere with our analyses. Cell lines expressing WT825 or mutant IL-4Rα-α-chains (Δ712 and Y713F) were sorted for comparable levels of GFP expression. The levels of surface receptor expression were confirmed by FACS analysis and correlated with that of GFP expression (Fig. 2A).

Previous reports on EPO signaling pathways suggested an important role of ITIM in terminating proliferative signals (29). To
SHP-1, SHP-2, Shc, and SHIP can associate with the ITIM of hIL-4Rα

Although IL-4Rα contains a putative ITIM, and this ITIM may regulate proliferation, the molecular mechanisms for this signaling remain unexplored. SHP-1, which has been demonstrated to bind to the ITIM motif of several receptors, could be coprecipitated with IL-4Rα (31). However, the nature of the association between IL-4Rα and SHP-1 is unclear. To determine whether the ITIM of IL-4Rα could associate with SH2-containing phosphatases such as SHP-1, SHP-2, and SHIP, the peptide corresponding to the ITIM of hIL-4Rα was synthesized. As shown in Fig. 3, tyrosine-phosphorylated peptides coprecipitated SHP-1 and SHIP in whole cell extracts prepared from unstimulated or IL-4-stimulated 32D/IRS-2 cells, whereas no interaction between the two phosphatases and nonphosphorylated peptides was observed. This is consistent with the tyrosine phosphorylation requirement for ITIM recruitment of SH2-containing phosphatases (17, 18). Two additional proteins on the anti-phosphotyrosine blot were identified as SHP-2 and anti-Shc Abs (data not shown).

SHP-1 has previously been shown to associate with the IL-4R (31). The data presented above suggest that SHIP can interact with the ITIM peptide sequence of hIL-4Rα. To verify the in vivo association of IL-4Rα ITIM with SHIP, coimmunoprecipitation experiments with whole cell extracts were performed. Human and murine IL-4R share significant homology in the region of the proposed ITIM (see Fig. 1). Upon IL-4 treatment, SHIP was specifically associated with murine IL-4R α-chain (Fig. 2B). Moreover, SHIP is coimmunoprecipitated with SHP-1 and Shc (Fig. 3). The association of SHP-1 and SHIP with the tyrosine-phosphorylated IL-4Rα ITIM, A, SHP-1 and SHIP bind the phosphorylated ITIM peptide of IL-4R α-chain. Whole cell lysates prepared from M12 cells (1 x 10⁷ cells) were incubated with nonphosphorylated (Y) and phosphorylated (pY) peptides coupled to agarose beads. Material adsorbed by peptides was resolved by 7% SDS-PAGE, transferred onto a nitrocellulose membrane, and subsequently immunoblotted with anti-phosphotyrosine, anti-SHIP, and anti-SHP-1 Abs. B, SHIP is coimmunoprecipitated with IL-4R α-chain by anti-IL-4R α Abs. Immunoprecipitations of lysates prepared from unstimulated and stimulated M12 were performed using anti-mIL-4Rα and normal rabbit serum (NRS). Immunoprecipitates were resolved by 7% SDS-PAGE and immunoblotted with anti-SHIP Ab. The same blot was stripped and reprobed with anti-mIL-4Rα Ab.

determine whether the IL-4Rα ITIM can regulate proliferative signals induced by IL-4, we measured the proliferation of 32D/IRS-2 cells expressing WT or ITIM-deficient IL-4Rα (Δ712 and Y713F) by [³H]thymidine incorporation assay. Fifty-three hours after IL-4 stimulation 32D/IRS-2 cells were gated and analyzed for surface expression of hIL-4R, biotinylated goat anti-mouse Ig and avidin-conjugated APC. GFP-positive 32D/IRS-2 cells expressing WT or ITIM-deficient hIL-4Rα were stained with anti-human IL-4R, mouse mAbs, followed by incubation with biotinylated goat anti-mouse Ig and avidin-conjugated APC. GFP-positive cells were gated and analyzed for surface expression of hIL-4R α-chains.

B. Proliferative responses of 32D/IRS-2 cells expressing WT, Δ712, or Y713F hIL-4Rα stimulated with various concentrations of hIL-4. A control, proliferation was estimated in the presence of 5% WEHI-3-conditioned medium as a source of IL-3. Each data point represents the mean ± SD of triplicate counts.

FIGURE 2. IL-4-induced proliferation of 32D/IRS-2 cells expressing WT and mutant hIL-4R α-chains. A, Surface expression of hIL-4Rα by 32D/IRS-2 cells expressing WT or ITIM-deficient hIL-4Rα. Cells were stained with anti-human IL-4R α mouse mAbs, followed by incubation with biotinylated goat anti-mouse Ig and avidin-conjugated APC. GFP-positive cells were gated and analyzed for surface expression of hIL-4R α-chains.

FIGURE 3. The association of SHP-1 and SHIP with the tyrosine-phosphorylated IL-4Rα ITIM. A, SHP-1 and SHIP bind the phosphorylated ITIM peptide of IL-4R α-chain. Whole cell lysates prepared from M12 cells (1 x 10⁷ cells) were incubated with nonphosphorylated (Y) and phosphorylated (pY) peptides coupled to agarose beads. Material adsorbed by peptides was resolved by 7% SDS-PAGE, transferred onto a nitrocellulose membrane, and subsequently immunoblotted with anti-phosphotyrosine, anti-SHIP, and anti-SHP-1 Abs. B, SHIP is coimmunoprecipitated with IL-4R α-chain by anti-IL-4R α Abs. Immunoprecipitations of lysates prepared from unstimulated and stimulated M12 were performed using anti-mIL-4Rα and normal rabbit serum (NRS). Immunoprecipitates were resolved by 7% SDS-PAGE and immunoblotted with anti-SHIP Ab. The same blot was stripped and reprobed with anti-mIL-4Rα Ab.
previous studies have demonstrated that the tyrosine phosphorylation of SHIP was enhanced with IL-4 stimulation (12, 32). Together, these data demonstrate that SHIP can associate with the IL-4R.

**IL-4 does not induce tyrosine phosphorylation of SHP-1 and SHP-2 in 32D cells**

The direct interaction of phosphatases with IL-4Rα ITIM suggests a contribution of these phosphatases to the ITIM-regulated proliferative response induced by IL-4. Previously, we and others have shown that SHIP is tyrosine-phosphorylated after stimulation by IL-4, and this SHIP phosphorylation was not affected by the mutation of Y713, suggesting the Y713 is not necessary for the recruitment of SHIP to the receptor (12, 32). Indeed, we have shown the direct association of SHIP and Jak1 in 293 cells, indicating SHIP can be recruited to the receptor via another mechanism (32).

We determined whether SHP-1 and SHP-2 could be tyrosine-phosphorylated in response to IL-4 stimulation in 32D/IRS-2 cells. As shown in Fig. 4A, SHP-1 was not tyrosine-phosphorylated in response to hIL-4 in 32D/IRS-2 cells expressing the different forms of hIL-4Rα. Western blotting demonstrated that SHP-2 was constitutively tyrosine phosphorylated in these cells. However, culture with hIL-4 did not alter the tyrosine phosphorylation of SHP-2 in these cells (Fig. 4B). Thus, alterations of IL-4-induced proliferation downstream of signaling initiated by IL-4Rα lacking the ITIM motif cannot be simply explained by the altered tyrosine phosphorylation of these two phosphatases.

**Activation of Stat6, but not Jak1 or IRS-2, is affected by mutation of Y713**

We next determined the effect of mutation in the IL-4Rα ITIM on the signaling components of the IL-4R complex. Binding of IL-4 to IL-4R causes receptor oligomerization and induces Jak1/3 tyrosine kinase activation. Both IRS-2 and Stat6 are recruited to the activated IL-4R complex and subsequently tyrosine phosphorylated upon IL-4 stimulation (33). Stat6 is involved in the IL-4-mediated gene activation (6). IRS-2 is required for IL-4-induced proliferation (9). We analyzed the effect of mutation of hIL-4Rα on Jak1 tyrosine phosphorylation, IRS-2, and Stat6 activation induced by hIL-4 using the cell lines described above.

Upon stimulation of hIL-4 for 20 min, similar levels of Jak1 tyrosine phosphorylation were observed in cells expressing WT825 or mutant Y713F, suggesting that initiating signaling events are not altered by the ITIM function (Fig. 5A). Similarly, IRS-2 becomes tyrosine phosphorylated to equal levels when hIL-4 is added to cells expressing either the WT or mutant receptor equally (Fig. 5B). In contrast, we found that IL-4 induced significantly (3.5-fold by analysis with a phosphorimager) increased tyrosine phosphorylation of Stat6 in cells expressing the mutant Y713F IL-4Rα compared with cells expressing the WT WT825.
receptor (Fig. 5C). Moreover, Stat6 binding activities were higher in mutant Y713F cells compared with WT825 cells and were detected up to 96 h after IL-4 stimulation in both cell lines (Fig. 5D). These results suggest that signaling through an IL-4R lacking the ITIM motif results in greater activation of Stat6.

The mechanism by which the IL-4Rα ITIM down-modulates IL-4-induced proliferation is still unclear. Because SHP-1, SHP-2, and SHIP associate with the IL-4Rα ITIM (Fig. 3A), it is possible that the IL-4Rα ITIM regulates IL-4-induced proliferation through the activities of one or more of these SH2-containing phosphatases. The increased tyrosine phosphorylation of Stat6 downstream of hIL-4Rα lacking the ITIM suggests that the tyrosine phosphatases SHP-1 and/or SHP-2 may play a role in regulating this process. SHP-1 has been shown to be recruited to the cytoplasmic tail of EPO-R and plays a major role in the down-modulation of proliferative signals induced by EPO (29). Upon EPO stimulation, SHP-1 specifically associates, via its SH2 domain, with phosphorylated tyrosine in the EPO-R ITIM. The recruited SHP-1 then mediates its regulatory function by dephosphorylating and inactivating Jak2. Indeed, some studies have suggested a role for SHP-1 in negative regulation of IL-4-R signaling (34, 35). In contrast, SHP-2 has been shown to be a positive regulator of signaling downstream of several receptors (36). In IL-4 signaling, it is possible that after recruitment to the phosphorylated ITIM one of these phosphatases dephosphorylates and thereby activates Stat6. Alternatively, one of these phosphatases could dephosphorylate and modulate the function of another regulator of Stat6 activation (e.g., suppressor of cytokine signaling).

Previous studies have demonstrated that SHP-1 is associated with IL-4-R in a stimulation-dependent manner (31, 37, 38). However, several reports have shown that neither SHP-1 nor SHP-2 is tyrosine phosphorylated in response to IL-4 stimulation. Although this could suggest these phosphatases are not activated in response to IL-4, the effect of tyrosine phosphorylation on enzymatic activity of SHP-1 and SHP-2 is still unclear (39, 40).

The importance of SHIP in IL-4 signaling is less clear. It has been proposed that SHIP could mediate an anti-proliferative effect by competing with Grb2-Sos complex for binding to Shc, resulting in a block in the Ras signaling pathway (41–43). On the other hand, the enzymatic activity of SHIP may be required for its regulatory function. Recently, we have demonstrated that SHIP can function as a positive regulator in IL-4-induced proliferation, and this requires the enzymatic activity of SHP (32). The possible recruitment of SHIP to a functional ITIM in the IL-4Rα chain and a positive role for SHIP in proliferation appear at odds. However, SHIP can bind to IL-4-R through other proteins, such as Jak1 (32, 44). The finding that cells expressing an IL-4Rα chain Y713 mutant are still capable of phosphorylating SHIP supports a model in which recruitment via the IL-4Rα chain ITIM may not be the only mechanism for SHIP recruitment to the activated IL-4R complex. Alternatively, SHIP may perform different functions if recruited to distinct portions of the receptor complex. Such differences could arise from the availability of different substrates to SHIP catalytic activity, secondary to either spatial or temporal differences.

A final possibility is that the functional consequence of mutating the ITIM is not due to the loss of binding of these phosphatases. This motif could be a docking site of another signaling molecule, not identified by these studies, which regulates IL-4-induced proliferation. Alternatively, the ITIM could function to alter the biology of the receptor itself by altering its position within the cell membrane or its ability to recycle to the surface after ligand binding. Previously, work has demonstrated that in order for cells to proliferate in response to IL-4 they must express either IRS-1 or IRS-2. Interestingly, studies using Stat6-deficient cells have shown that Stat6 also plays a role in IL-4-mediated cellular proliferation (45, 46). Stat6-deficient mice demonstrate decreased Th2-mediated immune responses and produce less IgE and IgG1 than controls (45, 47, 48). The relative contributions of the proliferative and differentiative functions of IL-4 to these defects are not clear. In this report we show that although tyrosine phosphorylation of IRS-2 by IL-4 stimulation was not affected by Y713 mutation (Fig. 5B), Stat6 was activated to a much greater extent by IL-4 stimulation of Y713 mutant cells compared with WT cells (Fig. 5, C and D). The stronger Stat6 tyrosine phosphorylation in 32D/IRS-2 expressing hIL-4Ra/Y713F (Fig. 5C) suggests that the tyrosine phosphatase could be involved in ITIM-mediated negative regulation. Although it is not yet clear how IL-4Rα ITIM regulates IL-4-induced proliferation in 32D/IRS-2 cells, the data presented here strongly suggest the presence of a functional ITIM at the C-terminus of hIL-4Rα-chains and thus demonstrate a new regulatory element in the IL-4 signaling pathway.

Several polymorphisms of the hIL-4Rα-chain have been linked to the development of allergic immune responses (49). The mechanism by which many of these alterations modulate IL-4 function in vivo remains unknown. Whether an alteration in proliferation, of the magnitude imparted by the loss of the ITIM motif, could alter the quality or quantity of an immune response awaits further study.

Acknowledgments

We thank Dr. Benjamin Neel for the anti-SHP-1 Ab, Dr. Jacalyn Pierce for the 32D/IRS-2 cell line, Dr. Gary Nolan for the FlNCX cell line, and Drs. William Paul and John Ryan for the WT hIL-4Ra plasmid.

References


