hTid-1, a Human DnaJ Protein, Modulates the Interferon Signaling Pathway*

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The Jak family of protein-tyrosine kinases are crucial for the signaling of a large number of different polypeptide ligands, including the interferons, many cytokines, erythropoietin, and growth factors. Through their interaction with receptors, the Jaks initiate a signaling cascade resulting in the activation of gene transcription and ultimately a cellular response to various ligands. In addition to their role in cellular signaling, alteration of Jak activity has been implicated in several disease states. In identifying Jak2-interacting proteins with the yeast two-hybrid system, we cloned the human homologue of the Drosophila melanogaster tumor suppressor gene lethal (2) tumorous imaginal discs, which encodes the protein Tid56. Drosophila Tid56 and its human homologue hTid-1 represent members of the DnaJ family of molecular chaperones. The TDI gene encodes two splice variants hTid-1S and hTid-1L. We confirmed the interaction between endogenous hTid-1 and Jak2 was shown in HEp2 cells. We further showed that hTid-1 interacts with the human interferon-γ (Hu-IFN-γ) receptor subunit IFN-γR2. In addition, using a chimeric construct where the extracellular domain of IFN-γR2 was fused to the kinase domain of Jak2, we showed that hTid-1 binds more efficiently to the chimera with an active kinase domain than to a similar construct with an inactive kinase domain. Additionally, the data demonstrate that hTid-1 isoforms as well as Jak2 interact with Hsp70/Hsc70 in vivo, and the interaction between Hsp70/Hsc70 and hTid-1 is reduced after IFN-γ treatment. Furthermore, both hTid-1S and hTid-1L can modulate IFN-γ-mediated transcriptional activity.

IFN-γ1 is a multifunctional cytokine that plays a variety of immunomodulatory and antineoplastic functions, including regulation of cell proliferation, cytotoxic differentiation, and apoptosis of T-lymphocytes. IFN-γ participates in immune surveillance and tumor suppression (1, 2). The biological activities of IFN-γ are mediated through a heterodimeric transmembrane receptor capable of activating the Jak-STAT pathway (3, 4). Tyrosine kinases of the Janus kinase (Jak) family play a crucial role in signal transduction via cytokine receptors (5). At present, the Jak family consists of four mammalian members Jak1, Jak2, Jak3, and Tyk2 (6). A single Jak homologue has been identified in Drosophila as the gene associated with hopscotch (hop) mutations (7). Jaks appear to be constitutively associated with the membrane-proximal intracellular domains of cytokine receptors and become rapidly phosphorylated after ligand binding and dimerization or oligomerization of the receptor chains. This is followed by a cascade of events generating cytokine-mediated gene transcription. Activated Jaks phosphorylate tyrosine residues at distal parts of the intracellular domains of the receptor chains, thereby generating docking sites for signal transducers and activators of transcription (STATs) and other intracellular signaling molecules (8). STATs, which are recruited to the specific phosphotyrosine-containing motifs located in the cytoplasmic part of the receptors, are in turn phosphorylated by activated Jaks at distinct tyrosine residues (3, 8, 9). Following phosphorylation STATs form homo- and heterodimers then translocate to the nucleus and bind to specific response elements of genes leading to their transcriptional activation (8, 10). In addition to their role in cytokine signaling, Jak kinases may play a more diverse role in mammalian physiology. The Jaks are activated in response to acute pressure load suggesting a role for Jak kinases in cardiovascular physiology (11). The Jaks have been linked to gliogenesis, consistent with a role for Jak kinases in central nervous system, and Jaks have been suggested to regulate potassium channels in response to prolactin (12–15). These observations support the hypothesis that Jak kinases have a variety of roles in signal transduction and possibly other events. Due to the involvement of Jaks in development and disease (16), it is of interest to identify proteins interacting with Jak kinases.

To identify novel proteins involved in Jak signaling, we employed the yeast two-hybrid system with Jak2 as a bait (17, 18). This approach led to the identification of a number of cDNA

1 The abbreviations used are: IFN-γ, interferon-γ; STAT, signal transducers and activators of transcription; Jak, Janus kinase; SMN, Spinal Motor Neuron; JBP2, Jak binding protein 2; HA, hemagglutinin; RT, reverse transcription; Hu-IFN, human IFN; DMEM, Dulbecco’s modified Eagle’s medium; DBD, DNA binding domain; AD, activation domain; KD, kinase domain; KDM, kinase domain mutant; GAS, gamma-activated sequence; PVDF, polyvinylidene difluoride.
clones interacting with Jak2: the Spinal Motor Neuron (SMN) cloners. The second round of PCR was performed with the product from the library, a phage DNA as the template and the SP6 and anti-Hsp70 (W27) (catalog number SC-24) were purchased from Santa

**APPLICATION EXPERIMENTS**

**Construction of a Plasmid Encoding GAL4-Jak2 and Screening for Jak2-interacting Proteins with the Two-hybrid System—Expression vectors for Jak kinases were obtained from Dr. James Ihle (St. Jude Children’s Research Hospital, Memphis, TN). The two-hybrid system vector pAS2, which contains the yeast tryptophan (TRP1)-selectable marker and a hemagglutinin (HA) tag, was provided by Stephan Ehrlich (27). The construction of the vector pAS2-Jak2 containing the GAL4 DNA binding domain fused to murine Jak2 was described by Pollack et al. (18).

**Cloning of the JBP2 cDNA**—The insert from plasmid p15M1 was approximately 2.6 kb in length. To isolate a longer clone, this insert was used as a probe to screen a human M426 cDNA library (28). From this cDNA library, a phage λ clone (15M1) was identified from which phagemid p15M1 containing a 2.6-kb insert was isolated. Because this clone did not contain an in-frame translation start site, PCR was used to obtain the 5’-region with DNA isolated from the M426 library (the source of the 15M1 cDNA) as the template. The first round of PCR was performed with 10 ng of whole λ phage DNA as the template and the SP6 and 15-5-2 (5’-GGCTGGCATTTCGAGGCAC-3’) oligonucleotides as primers. The second round of PCR was performed with the product from the first round as the template with the oligonucleotides SP6 and 15-5-3 (5’-GGAGGCAGATCAAAATGAAGG-3’) as primers. The PCR products were cloned into the vector pCR2.1 (Invitrogen, San Diego, CA). The nucleotide sequences of the modified regions of all constructs were verified in their entirety by DNA sequencing.

**Construction of Mammalian Vectors for the Expression of TID1—**

**Antibodies**—Rabbit polyclonal anti-Jak2 (catalog number SC-294) and anti-cytochrome C (H-104, catalog number SC-7159), monoclonal anti-Hap70 (W27) (catalog number SC-24) were purchased from Santa Cruz Biotechnology. Monoclonal anti-phosphotyrosine antibody was from Sigma Chemical Co. (catalog number P3030). Monoclonal antibody against Hu-IFN-γ (CD 119) was a gift from PBL Biochemical Laboratories (New Brunswick, NJ; catalog number 21585-1). Anti-FLAG antibody M2 was from Sigma (catalog number F-3165). Anti-hTid1 monoclonal R813 hybridoma supernatant was kindly provided by Karl Munger (Harvard Medical School).

**Immunoprecipitation, Western Blotting—**Cells were starved overnight in serum-free media followed by transfection and subsequently stimulated with Hu-IFN-γ (1000 units/ml) for 40–60 min at 37 °C. Extracts were made from dishes of adherent cells as described by Pollack et al. (18). For immunoprecipitation, lysates were incubated with appropriate antibody for 1 h overnight at 4 °C with rocking. Immunoprecipitation and Western blotting was performed as described previously (18). Blots were developed with Renaissance Western blot chemiluminescence reagent plus (PerkinElmer Life Sciences, catalog number NEL105).

**Subcellular Fractionation—**HEp2 cells were washed in phosphate-buffered saline, scraped into sucrose buffer containing 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.25 mM sucrose, and protein inhibitor mixture (1 μg/ml leupeptin, 2 μg/ml antipain, 10 μg/ml benzamidine, 10 μg/ml aprotinin, 1 μg/ml leuprin, 1 μg/ml leuprin). A second PCR reaction was performed with 1 μl of the first reaction as template and cloned into the vector pBluescript SK(+) (30) as described previously (18).

**Cells, Media, and Transfection—**Cos-1 and HEp2 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen) and 10% Cosmic calf serum (HyClone) in 5% CO2 environment at 37 °C. Plates (100 mm) containing 70–80% confluent COS-1 cells were transiently transfected with 5–6 μg of total expression vector by the DEAE-dextran procedure with dimethyl sulfoxide shock (33).
RESULTS

Identification of Jak2-interacting Proteins in the Yeast Two-hybrid System—To screen for Jak2-interacting proteins with the yeast two-hybrid system, the murine Jak2 cDNA was cloned into the vector pAS2 (27). This fused the GAL4 DNA binding domain (GAL4_DBDB) to amino acids 19–1129 of the murine Jak2 cDNA. The expected size of the GAL4_DBDB-Jak2 fusion protein was about 139 kDa. Expression of this protein was confirmed by immunoprecipitation and Western blot analysis (data not shown). The yeast strain Y190 was cotransformed with the plasmid encoding the GAL4_DBDB-Jak2 fusion protein and an Hela cell GAL4 activation domain (GAL4_AD) library created for use in the yeast two-hybrid system (18). The yeast strain Y190 contains two reporter genes (lacZ and HIS3) whose transcriptional activation indicates an interaction between the two GAL4 fusion proteins. Transformants were first selected for expression of the HIS3 reporter gene by plating onto media lacking histidine. Histidine prototrophs were selected and subsequently tested for activation of the lacZ reporter gene with the β-galactosidase paper filter assay (18). After screening 1.6 × 10⁶ transformants, 143 histidine prototrophs were selected for the β-galactosidase paper filter assay. Twenty-eight of those assays showed evidence of β-galactosidase activity. To determine whether the interactions were dependent on the expression of the GAL4_DBDB-Jak2 fusion protein, a mating assay was performed as previously described (27). After removing the pAS2-Jak2 plasmid, yeast containing only the library plasmids were mated with yeast containing the pAS2-Jak2 plasmid, and loss of this plasmid was confirmed by testing for tryptophan auxotrophy and yeast containing only the library plasmids were mated to yeast of the opposite mating type, which contained several “decoy” plasmids encoding various GAL4_DBDB fusion proteins fused to lamin, CDK2, or SNF1 as seen in the figure. Following the growth of diploids on selective media, the expression of β-galactosidase was assayed by filter paper assay. The original interaction was retested by including yeast containing the pAS2-Jak2 plasmid in this mating assay.

for the enzyme argininosuccinate synthetase. This enzyme is a component of the urea cycle and the arginine:carboxylate cycle (21, 22).

Five clones represented the SMN cDNA. The SMN gene was first identified as a gene involved in the pathogenesis of spinal muscular atrophy, a common fatal autosomal recessive disease characterized by the degeneration of lower motor neurons (19). Subsequent reports demonstrated that the SMN protein interacts with Bcl-2 and that coexpression of the SMN protein with Bcl-2 enhances the anti-apoptotic effect of Bcl-2 against Bax-induced or Fas-mediated apoptosis (36). An interaction between the SMN protein and Jak2 may be one mechanism through which Jak2 exerts its anti-apoptotic effects (37). The SMN gene was found in five independent clones during the screening for Jak2-interacting proteins (Table I). Four of these five clones appear to be missing exon 7 as determined by PCR analysis around this region. Such a splice variant has been reported to have a dominant-negative effect with respect to the anti-apoptotic activity of SMN (36). One clone, within the library plasmid called p15-3A, contained an insert of 2.15 kb and represented a human homologue of the D. melanogaster protein Tid56, which is encoded by the gene t(2)tid (23, 25, 38).

Isolation of a cDNA Encoding a Human Homologue of Drosophila Tid56—The library plasmid p15-3A, with a 2.15-kb insert, encoded an open reading frame that had homology to Drosophila Tid56. This open reading frame began with a region homologous to the middle of the Tid56 sequence and, therefore, represented a partial cDNA clone. To isolate a longer clone corresponding to this cDNA, a human M426 cDNA library was screened, resulting in the isolation of cDNA clone 15M1, with a
2.6-kb insert. This cDNA referred to as JBP2 or Jak binding protein 2 encoded a protein of 449 amino acids. Because clone 15M1 contained no 5’ translation initiation codon, PCR was used to amplify additional 5’ sequences from the human cDNA library with both insert and library oligonucleotides as primers. The largest fragment, 15N8 (see “Experimental Procedures”) resulted in the identification of an additional 54 nucleotides 5’ upstream of the beginning of the 15M1 cDNA and included an in-frame translation-initiation codon four amino acid residues upstream from the first amino acid encoded by the 15M1 sequence. The full sequence of the JBP2 cDNA was constructed as a contig from plasmids 15A-3A, 15M1, and 15N8 (GenBank™ accession number AF411044).

Schilling et al. (25) isolated a cDNA encoding a human homologue of Tid56 called TID1 from a two-hybrid system screen for proteins interacting with the human papillomavirus type16 (HPV-16) E7 protein. The TID1 cDNA encodes a protein similar to JBP2. The amino acid sequences of these two clones differ at amino acid position 75 with JBP2 encoding a tyrosine and the TID1 an asparagine. It was also reported that both JBP2 and TID1 contain a different C terminus than the hTid-1 protein. In following up their work, Syken et al. (26) reported that TID1 encodes two splice variants, a long form, hTid-1L (43 kDa), and a short form, hTid-1S (40 kDa). The short form was identical to the JBP2 sequence reported here except for the tyrosine-asparagine substitution. In subsequent sequence analysis of the RT-PCR product generated from RNA isolated from a variety of cell lines, we conclude that there is an allelic polymorphism consisting of double-nucleotide substitution (C to T) and (T to A) at positions 207 and 223 with respect to the AUG start codon. The second substitution (T to A) leads to a change from tyrosine to asparagine. It was also reported that both TID1 forms were mitochondrial proteins, with hTid-1L stimulating apoptosis and hTid-1S suppressing apoptosis in response to DNA-damaging agents such as mitomycin C or tumor necrosis factor-α (26).

**Fig. 2. Distribution of hTid-1 in leukemia cell lines.** Cell lysates from human leukemia lines Thp1, RPMI8402, Raji, Reh, Jurkat, U937, and peripheral blood mononuclear cells from healthy donor was analyzed by SDS-PAGE and transferred to a PVDF membrane and probed with anti-hTid-1 antibody. Bands corresponding to hTid-1L and hTid-1S are indicated by arrows on the left. A lower molecular weight band observed in Thp1 and U937 is indicated by the arrow on the right.

### TABLE I

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<td>5</td>
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The ten library plasmids that activated the lacZ reporter gene specifically with Jak2 in the two-hybrid system were rescued and sequenced. The original clone designations are shown in the column. Clone names and the number of independent clones isolated are shown in the left column labeled Number of Isolates. The identity of the insert is shown in the right column.
**hTid-1 Modulates Interferon Signaling**

**Fig. 3. Interaction of Jak2 with hTid-1 and hTid-1**.<br>COS-1 cells were transfected with Jak2 expression vector (3 μg) and pEF3-hTid-1 or pEF3-hTid-1L (1 μg). The total amount of DNA was adjusted to 5 μg with the vector DNA, pcDEF3. Cell extracts were prepared after 48 h following overnight serum starvation followed by stimulation with IFN-γ (1000 units/ml) for 1 h when indicated. 500 μl of extract was incubated with appropriate antibodies and immunoprecipitated overnight at 4 °C followed by incubation with agarose A/G plus beads. Immunoprecipitates were analyzed by SDS-PAGE and immunoblotted with different antibodies. A-C, hTid-1 immunoprecipitate blotted with anti-Jak2 antibody or anti-hTid-1 or anti-P-Tyr, respectively. D, anti-Jak2 immunoprecipitate was probed with anti-Jak2 antibody. E, anti-Jak2 immunoprecipitates were immunoblotted with anti-P-Tyr.

**Fig. 4. Interaction of endogenous hTid-1 and Jak2 in HEp2 cells.** HEp2 cells were either left untreated or treated with IFN-γ (1000 units/ml) for 1 h. Lysates were prepared as described under “Experimental Procedures.” Immunoprecipitation with anti-hTid-1 antibody was performed and analyzed by SDS-PAGE and transferred to a PVDF membrane and blotted with hTid-1 and Jak2-specific antibodies.

**Fig. 5. Stable interaction between Jak2, hTid-1, and Hu-IFN-γ R2.** COS-1 cells were transfected with the IFN-γ-R2 expression vector alone or in combination with Jak2 or hTid-1, or hTid-1L expression vectors (1 μg each). Cell extracts were prepared after 48 h following overnight serum starvation of cells followed by stimulation with IFN-γ (1000 units/ml) for 1 h when indicated. Immunoprecipitation with hTid-1-specific antibody and analysis of immune complexes were performed as described in detail in Fig. 3. hTid-1 immunoprecipitates were blotted with Hu-IFN-γ-R2-specific antibody (A, upper and B, upper), hTid-1-specific antibody (A, lower and B, middle), or Jak2-specific antibody (B, lower).

Hsc70, cellular lysates from HEp2 cells with and without treatment with IFN-γ were immunoprecipitated with anti-hTid-1 antibody. Coimmunoprecipitation of Hsp70/Hsc70 in HEp2 cells with anti-hTid-1 antibody indicate that these two proteins interact in vivo (Fig. 6A). In addition, Hsp70/Hsc70 antibody immunoprecipitated hTid-1 as a function of time after IFN-γ treatment as shown in Fig. 6B (upper panel). The level of Hsp70/Hsc70 protein in both untreated and IFN-γ-treated immunoprecipitates from HEp2 cells is shown as a function of time after IFN-γ treatment in the lower panel of Fig. 6B. The interaction between hTid-1 isoforms and Hsp70/Hsc70 was reduced in cells treated with IFN-γ.

We further examined whether Hsp70/Hsc70 can bind to Jak2 and conceivably serve as a substrate for the Hsp70/Hsc70 chaperone. Extracts from HEp2 cells either treated with IFN-γ or left untreated were immunoprecipitated with anti-Jak2 antibody and immunoblotted with anti-Hsc70 antibody. As shown in Fig. 6C, anti-Jak2 antibody coimmunoprecipitated Hsp70/Hsc70 in HEp2 cells treated or untreated with IFN-γ.

**Subcellular Localization of hTid-1 in HEp2 Cells—**To determine whether mature hTid-1 is also located outside mitochondria, subcellular fractionation of HEp2 cells was performed. Equal amounts of protein from the nuclear, mitochondrial, and cytoplasmic fractions were tested in Western blotting with anti-hTid-1 antibody. Anti-cytochrome C antibody was used to confirm the purity of the mitochondria-enriched fractions (Fig. 7A). Although hTid-1 was predominantly located in mitochondria, mature hTid-1 proteins were detected in the nucleus and cytoplasm (Fig. 7B). Coimmunoprecipitation of different fractions with anti-Jak2 antibody revealed the Jak2-hTid-1 interaction was detectable mainly in the cytoplasm (Fig. 7C). Moreover, interaction of hTid-1 with Hsp70/Hsc70 (Fig. 6A), which localizes in cytoplasmic and nuclear compartments of the cell,
hTid-1 Modulates Interferon Signaling

To examine whether an active Jak2 kinase domain was needed for the Jak2-hTid-1 interaction, a chimeric construct, pEF3-FLγR2/Jak2KDM with the intracellular domain of IFN-γR2 replaced with the kinase domain of Jak2, was made. This construct also has a FLAG tag at the N-terminus for easy detection. A second construct (pEF3-FLγR2/Jak2KDM) identical to the first one except that the Jak2 kinase domain was inactive due to a Lys-882→Glu mutation was constructed. These two constructs were transiently transfected in COS-1 cells alone or in combination with either hTid-1S or hTid-1L, expression vectors. Lysates from the transfected cells were immunoprecipitated with anti-FLAG antibody M2. A significantly higher amount of hTid-1 was recovered from cells that were transfected with the chimera containing the active kinase domain (pEF-FLγR2/Jak2KD) compared with the cells transfected with chimera containing the inactive kinase domain (pEF-FLγR2/Jak2KDM) (Fig. 8, upper panel). Both unprocessed, presumably cytoplasmic, and mature mitochondrial hTid-1S and hTid-1L are indicated by arrows (upper panel).

hTid-1 Modulates IFN-γ-mediated Transcription—After IFN-γ binds to its receptor, the phosphorylation of Jak kinases, then phosphorylation of STAT1, dimerization, and nuclear translocation of the latent cytoplasmic STAT1 transcription factor, take place sequentially. The activated STAT1 binds to the gamma-activated sequence (GAS) element to activate gene transcription. To determine the ability of hTid-1 to affect expression of genes induced by IFN-γ, a luciferase reporter gene (GAS-WT) was tested in a cotransfection assay. Lysates from COS-1 cells expressing the reporter plasmid alone or coexpressing hTid-1S or hTid-1L were examined for luciferase activity. Overexpression of hTid-1S or hTid-1L reduced luciferase activity.

suggests that TID1 isoforms have additional biological functions outside of the mitochondria; its biological activities may be dictated by subcellular location and its Hsp70 partner (39).

hTid-1 Binds to the Active Kinase Domain of Jak2—To examine whether an active Jak2 kinase domain was needed for the Jak2-hTid-1 interaction, a chimeric construct, pEF3-FLγR2/Jak2KDM with the intracellular domain of IFN-γR2 replaced with the kinase domain of Jak2, was made. This construct also has a FLAG tag at the N-terminus for easy detection. A second construct (pEF3-FLγR2/Jak2KDM) identical to the first one except that the Jak2 kinase domain was inactive due to a Lys-882→Glu mutation was constructed. These two constructs were transiently transfected in COS-1 cells alone or in combination with either hTid-1S or hTid-1L, expression vectors. Lysates from the transfected cells were immunoprecipitated with anti-FLAG antibody M2. A significantly higher amount of hTid-1 was recovered from cells that were transfected with the chimera containing the active kinase domain (pEF-FLγR2/Jak2KD) compared with the cells transfected with chimera containing the inactive kinase domain (pEF-FLγR2/Jak2KDM) (Fig. 8, upper panel). Both unprocessed, presumably cytoplasmic, and mature mitochondrial hTid-1S and hTid-1L are indicated by arrows (upper panel).

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COS-1 cells were transfected with reporter plasmid GAS-WT (+4900) plus various amounts of hTid-1 expression plasmids (0, 1, 2, 4 μg) as indicated. The total amount of DNA transfected was kept constant at 6 μg with the empty vector. 48 h following transfection, cells were treated with IFN-γ (+1000 units/ml) as indicated. Extracts were prepared, and luciferase assay was performed with equal amounts of protein in each sample. The background activity was subtracted from the IFN-γ-induced luciferase activity and plotted as a function of the concentration of transfected hTid-1 expression plasmids. Similar hTid-1-mediated inhibition of luciferase activity was observed in four independent experiments.

Luciferase activity induced by IFN-γ. Luciferase activity was reduced as the quantity of hTid-1S and hTid-1L vectors transfected into cells was increased (Fig. 9). This result demonstrates that hTid-1S and hTid-1L can inhibit the action of IFN-γ.

**DISCUSSION**

Jak kinases are crucial for signal transduction via the Jak-STAT pathway (2–5, 8, 41). In addition to their interactions with cytokine receptor chains, Jak kinases are known to interact with other signaling proteins. Jak2 interacts with SHPTP1 (42, 43), SHPPT2 (44), PP2A, phosphatidylinositol 3-kinase, Yes (45), Shc (46), Syk (47), Grb2 (48), the angiotensin II AT1 receptor (49), and the serotonin 5-HT2A receptor (50). The ability to interact with diverse proteins underscores the complex role of Jak2, which is activated by a majority of ligands that utilize the Jak-STAT pathway. The physiological roles for these interactions have not been fully characterized, nevertheless, they suggest that the Jaks play a role in other pathways and/or facilitate cross-talk between signaling pathways.

Jak2 is required for the proliferative response to many growth factors such as erythropoietin and growth hormone. A kinase-deficient Jak2 can block an erythropoietin-induced mitogenic signal (31). Hyperactivation of Jak2 has been seen in a number of malignancies, and inhibition of Jak activity has been shown to reverse the malignant phenotype of leukemic cells (51–53). Similarly, mutations causing overactivity of the Jak homologue *hopscotch* have been reported to cause a leukemic-like syndrome in *Drosophila* (54, 55). Consistent with their functional and sequence homology, mutation of homologous residues in *hopscotch* and Jak2 cause both proteins to become hyperactive (56). These reports suggest that Jak2 activity is involved in the control of proliferation and that uncontrolled activation of Jak2 causes the loss of proliferative control.

We used the yeast two-hybrid system to screen an HeLa cell library to identify novel Jak2-interacting proteins. One protein identified in this screen, hTid-1, has high homology to Tid56, the protein encoded by the *D. melanogaster* tumor suppressor gene *l(2)tid* (23). Tid56 and hTid-1 have 65.8 and 54.9% amino acid similarity and identity, respectively, and thus have been well conserved through evolution. Schilling et al. (25) cloned *TID1* cDNA during a screen for proteins, which interact with the human papilloma virus type 16 E7 oncoprotein. Our clone is identical to an alternatively spliced form of *TID1* designated as hTid-1G except for an Asn to Tyr change at amino acid 75. The motifs conserved between Tid56 and hTid-1 include the N-terminal J domain containing the highly conserved HPD tripeptide, a glycine/phenylalanine-rich region and four CXGRL repeats and a less conserved C-terminal region (23, 25, 26).

*Drosophila* Tid56 and its human homologue hTid-1 represent members of the DnaJ family, which function as molecular chaperones. DnaJ proteins contain a highly conserved tetrahelical domain that binds to the DnaK proteins and their eukaryotic homologues, Hsp70 proteins, and thereby regulate their activity and confer substrate specificity to Hsp70 proteins. In *Drosophila*, Tid56 is involved in the regulation of cell growth and death as evidenced by the hyperproliferative phenotype of the Tid56 mutant embryo (23). Interestingly, the Jak homologue *hopscotch* in *Drosophila* is required for the proliferation of diploid imaginal cells (57). Because mutations in Tid56 and the Jak2 homologue *hopscotch* confer similar phenotypic effects in *Drosophila*, it is possible that Tid56 is a modulator of Jak2 activity. A common link between these two pathways is likely.

We have shown that two splice variants of *TID1*, hTid-1S and hTid-1L, interact with Jak2 in vivo in COS-1 cells transfected with corresponding cDNAs. Interaction between the endogenous Jak2 and hTid-1S or hTid-1L was demonstrated in human HEp2 cells, and hTid-1 remains associated with Jak2 after IFN-γ treatment when dimerization and oligomerization of receptor chains occur following phosphorylation of Jak2. This observation prompted us to test the hypothesis that hTid-1 forms a complex with Jak2 and IFN-γR2. In cells transfected with corresponding cDNAs, we observed that Jak2 and IFN-γR2 coimmunoprecipitated with hTid-1. Interaction between hTid-1 and Hsp70/Hsc70 molecular chaperones were shown in HEp2 cells (Fig. 6). This interaction is significantly reduced after IFN-γ treatment. Hsp70 family members and their associated DnaJ chaperones mediate a number of cellular functions such as folding of newly synthesized polypeptides, the translocation of proteins across membranes, and assembly of multimeric protein complexes. Recently, DnaJ chaperones were shown to be components of intracellular signaling pathways linked to cell survival and growth regulation. Studies in a variety of systems involving receptors, protein-tyrosine kinases, and chaperone proteins emphasized the physiological relevance of such interactions. Genetic studies in yeast have demonstrated that binding of steroid receptors, the dioxin receptor, and some protein kinases to Hsp90 is critical for their signal transduction *in vivo* (58). The interferon-induced double-stranded RNA-activated protein kinase is inhibited by p58IPK, which contains a DnaJ motif (59).

Based on our observations, we propose a model of hTid-1-mediated modulation of IFN-γ signaling (Fig. 10). We propose that Jak2 and γR2 associate with hTid-1 and Hsp70/Hsc70 to form a complex, hTid-1 recruits Hsp70/Hsc70 to the receptor complex, and Jak2 may be a substrate for Hsp70/Hsc70. We propose that hTid-1 acts as a chaperone causing a conformational change in Hsp70/Hsc70 that allows it to interact with Jak2. The interaction of Hsp70/Hsc70 with Jak2 then inhibits the kinase activity of Jak2. It has been proposed that the maintenance of proper protein intermediate states might be important for the normal function of many substrate proteins, particularly those that participate in the assembly of multimeric complex structures and those that participate in signaling pathways (40). Upon IFN-γ treatment, Hsp70/Hsc70 and then hTid-1 are released from the complex thereby activating the kinase function of Jak2. Because the overexpression of hTid-1 inhibits IFN-γ-mediated signaling, we envision that hTid-1 is released from the Jak2-IFN-γR2 complex to initiate signal
transduction. Jak2 autophosphorylation in response to IFN-γ remains unaltered in COS-1 cells overexpressing h-Tid-1 as compared with the control (Fig. 3E). Therefore, we propose that inhibition of IFN-γ-induced luciferase activity by h-Tid-1 is related to the interference of Jak2-mediated receptor phosphorylation or STAT-1 phosphorylation. We observed that the Hsp70/Hsc70 and Jak2 interaction is not affected by IFN-γ treatment (Fig. 6B), a result that is likely due to the fact that Hsp70/Hsc70 interacts with Jak2 that is not part of the IFN-γ receptor complex.

The Jak-STAT signaling pathway can induce proliferation and differentiation and is crucial for the proper growth and development of mammalian tissues, particularly those of hematopoietic lineages. Moreover, hyperactivation of Jak2 is implicated in leukemia. Therefore, Jak2 activation needs to be tightly regulated. Our data suggest that h-Tid-1 is a negative modulator of the Jak-STAT pathway. To our knowledge this is the first report of a human DnaJ protein interacting with the Jak2 and IFN-γ receptor complex. Further understanding of the physiological significance of Jak2, h-Tid-1, and the Hsp70/Hsc70 interactions could shed light on the effects of h-Tid-1 (Tid56) in tumor suppression and cell growth.

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REFERENCES

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hTid-1 Modulates Interferon Signaling