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An Unusual Insertion in Jak2 Is Crucial for Kinase Activity and Differentially Affects Cytokine Responses

Claude Haan,1* Daniela C. Kroy,1† Stefan Wüller,†‡ Ulrike Sommer,‡ Tanja Nöcker,‡ Catherine Rolvering,* Iris Behrmann,* Peter C. Heinrich,†§ and Serge Haan2*†

The Janus kinases, Jaks, constitutively associate with the cytoplasmic region of cytokine receptors and play an important role in a multitude of biological processes. Jak2 dysfunction has been implicated in myeloproliferative diseases and leukemia. Although Jak2 was studied extensively for many years, the molecular mechanism of Jak activation upon cytokine stimulation of cells is still incompletely understood. In this study, we investigated the importance of an unusual insertion located within the kinase domain in Jak2. We found that the deletion of this insertion, which we named the Jak-specific insertion (JSI), totally abrogates Jak2 autophosphorylation. We further point mutated four residues within the JSI that are conserved in all Jak family members. Three of these mutants showed abrogated or reduced autophosphorylation, whereas the fourth displayed increased autophosphorylation. We found that the phosphorylation state of these mutants is not influenced by other domains of the kinase. Our data further suggest that the JSI is not required for the negative regulation of kinase activity by the suppressor of cytokine signaling proteins, SOCS. Most importantly, we show that mutations in this region differentially affect IFN-γ and erythropoietin signal transduction. Taken together, the dramatic effects on the phosphorylation status of Jak2 as well as the differential effects on the signaling via different cytokines highlight the importance of this unusual region for the catalytic activity of Jaks. The Journal of Immunology, 2009, 182: 2969–2977.

Protein kinases are involved in many biological pathways and deregulation of their kinase activity is observed in many diseases. In recent years, many reports on the three-dimensional structures of protein kinases have given valuable insights into the mechanisms underlying the regulation of kinase activity (1, 2). Although these studies revealed a high degree of conformational plasticity of protein kinase domains between the inactive and active state, the basic protein kinase fold is highly conserved.

Jaks are cytoplasmic kinases that constitutively associate with the cytoplasmic region of cytokine receptors. They activate transcription factors of the STAT family and play a pivotal role in a variety of biological processes such as cell growth, differentiation, and survival as well as in hematopoietic and immune responses (3, 4). Jak2 contains a 4.1/ezrin/radixin/moesin (FERM)3 domain, a potential SH2 domain, a kinase-like (or pseudokinase) domain, and a classical tyrosine kinase domain. Although Jaks have been studied extensively for many years, their mechanism of activation upon cytokine stimulation is still incompletely understood. Jak2 recently came into the focus of interest after a somatic mutation in Jak2 (Jak2-V617F) was identified in many patients suffering from myeloproliferative disorders (5, 6). This mutation leads to a cytokine-independent activation of Jak2 and downstream signaling proteins. Jak2 is involved in the signal transduction of many cytokines such as erythropoietin (Epo), IFN-γ, IL-3, growth hormone, thrombopoietin, and others (7–13). Depending on the cytokine/cytokine receptor system, signal transduction can involve either two Jak2 molecules or Jak2 paired with Jak1 or Tyk2, respectively. Upon treatment of cells with Epo, the ligand recruits two Epo receptor chains, each associated with Jak2, whereas IFN-γ stimulation triggers a heteromorphic receptor complex composed of IFN-γRα and IFN-γRβ, which are associated with Jak1 and Jak2, respectively. Using a molecular modeling approach, we detected an unusual insertion in the kinase domain of Jak2 located in close proximity to the catalytic cleft that we designated Jak-specific insertion (JSI). This region was also highlighted in the published crystal structures of the Jak2 and Jak3 kinase domains and termed “Lip” in the context of Jak2 (14, 15). In the present study, we investigated the importance of this unusual insertion in the context of Jak2. We find the JSI/Lip to be crucial for kinase activity and report that mutations in this region can differentially affect signal transduction via different cytokines.

Materials and Methods

Alignment of kinase domains, structure representation, and molecular modeling

For the graphic representation of protein structures, the programs PyMOL (16) and Ribbons (17) were used. The Brookhaven Protein Data Bank entry code for the Jak2 structure is 2b7a. The initial alignment of the kinase domain sequences was performed by the use of the BLAST (basic local alignment search tool) program (18). Modifications were then introduced to meet the structural requirements derived from the solved structures. The

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Generation of the Jak2 deletion construct ΔJSI

Generation of the Jak2 deletion construct ΔJSI and the F1061A mutant was performed using standard PCR techniques. M1062A, M1064A, I1065A, and L87A/M88A were generated using the QuikChange kit (Stratagene) following the manufacturer’s recommendations. The various constructs were transferred to the different vectors pSVL-Jak2, pcDNA5/FRT/TO-Jak2 (19), pcDNA5/FRT/TO-YFP-Jak2, and pEF-eYFP-Jak2-H1Iwt (20) using standard techniques. For the generation of pcDNA5/FRT/TO-YFP-Jak2, YFP-Jak2 was cloned from pcDNA5/HA-EpoR (human) construct was provided by Dr. S. Constantinescu (Ludwig Institute for Cancer Research, Brussels, Belgium). pgV-CIS-promoter-Luc and pEF-FLAG/SOC5-1 or SOCS-3 (where CIS is cytokine-inducible SH2-containing protein, Luc is luciferase, and SOCS is suppressor of cytokine signaling) were provided by Dr. A. Yoshimura (Kyushu University, Fukuoka, Japan) and Dr. D. J. Hilton (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia). The pGL2-IRF1-kLuc construct contains the STAT1-responsive element of the IRF1 promoter. For reporter gene analysis, a β-galactosidase expression plasmid, pCH110 (Pharmacia), was used. Detailed information on the cloning procedures is available upon request.

Reporter gene analysis

Cells were transiently transfected with 0.25 μg of the Luc reporter-gene constructs (pgV-CIS-promoter-Luc or pGL2-IRF1-kLuc) together with the respective SOCS expression plasmid or empty vector using Superfect reagent (Qiagen). Jak2 expression was induced for 24 h and subsequently the cells were stimulated with the respective cytokine for 24 h. Cell lysis and Luc assays were conducted using the Promega Luc assay system as described by the manufacturer. Luc activity values were normalized to transfection efficiency monitored by cotransfection of a β-galactosidase expression vector (0.2 μg of pCH110lacZ; Amersham Biosciences). Experiments were performed in triplicate.

Cell culture and transfection

γ2A cells (human fibrosarcoma cells provided by Dr. I. M. Kerr, Cancer Research UK, London, U.K.), HEK-FRT (where HEK is human embryonic kidney and FRT is Flp recombinase target) cells (Flp-In T-REx-293 cells; Invitrogen), and COS-7 cells (simian kidney cells, American Type Culture Collection number CRL1619) were maintained in DMEM (Invitrogen). All media were supplemented with 10% FCS, 100 μg/mL streptomycin, and 60 mg/mL penicillin. HEK-FRT cell lines stably expressing Jak2 constructs and reconstituted γ2A cells were cultured with 250 μg/mL hygromycin. Cells were grown at 37°C in a water-saturated atmosphere at 5% CO2. Transient transfection of COS-7 and γ2A cells (∼1 × 106 cells) was conducted using FuGENE 6 (Roche) or Superfect (Qiagen) according to the manufacturer’s recommendations. HEK-FRT cells stably expressing eYFP-Jak2, eYFP-Jak2-JSI, eYFP-Jak2-F1061A, eYFP-Jak2-M1062A, eYFP-Jak2-M1064A, and eYFP-Jak2-I1065A as well as γ2A-FRT-EpoR cells (where eYFP is enhanced yellow fluorescent protein and WT is wild type) reconstituted with Jak2 and the various mutants were generated using the Flp-In T-REx System from Invitrogen according to the manufacturers’ recommendations. The expression of eYFP-tagged Jak2 proteins was induced by incubating the cells with 10 μg/mL doxycycline for 12 h unless otherwise stated. IFN-γ and Epo were obtained from PeproTech. Cells were stimulated with 1000 U/ml IFN-γ or 5–10 μg/mL Epo for the times indicated.

Constructs

Generation of the Jak2 deletion construct ΔJSI and the F1061A mutant was performed using standard PCR techniques. M1062A, M1064A, I1065A, and L87A/M88A were generated using the QuikChange kit (Stratagene) following the manufacturer’s recommendations. The various constructs were transferred to the different vectors pSVL-Jak2, pcDNA5/FRT/TO-Jak2 (19), pcDNA5/FRT/TO-YFP-Jak2, and pEF-eYFP-Jak2-H1Iwt (20) using standard techniques. For the generation of pcDNA5/FRT/TO-YFP-Jak2, YFP-Jak2 was cloned from pcDNA5/HA-EpoR (human) construct was provided by Dr. S. Constantinescu (Ludwig Institute for Cancer Research, Brussels, Belgium). pgV-CIS-promoter-Luc and pEF-FLAG/SOC5-1 or SOCS-3 (where CIS is cytokine-inducible SH2-containing protein, Luc is luciferase, and SOCS is suppressor of cytokine signaling) were provided by Dr. A. Yoshimura (Kyushu University, Fukuoka, Japan) and Dr. D. J. Hilton (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia). The pGL2-IRF1-kLuc construct contains the STAT1-responsive element of the IRF1 promoter. For reporter gene analysis, a β-galactosidase expression plasmid, pCH110 (Pharmacia), was used. Detailed information on the cloning procedures is available upon request.

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cleft. It also has the fewest contacts with neighboring amino acids (Fig. 1C and Table I). As expected, upon mutation of the JSI residues to alanine, hydrophobic contacts with surrounding residues are lost or reduced for all of the investigated residues (Table I). The residue whose mutation affects contacts with neighboring amino acids least is Met1062. The most important structural role can be attributed to Phe1061.

Mutations within the JSI affect Jak2 kinase activity

To monitor mutant Jak2 activity, we first used an overexpression assay and studied Jak2 autophosphorylation (Fig. 2B). We found that the ΔJSI deletion mutant as well as the F1061A and I1065A mutants failed to show significant autophosphorylation. M1062A and M1064A displayed autophosphorylation, although to different extents. Most interestingly, the M1062A mutant showed increased autophosphorylation compared with WT protein. Mutation of M1064A resulted in decreased autophosphorylation.

The differential phosphorylation status of the mutants is independent of contacts with other Jak2 domains

As one of our original assumptions was that the JSI could serve as docking site for the negatively regulating kinase-like domain (JH2 domain), we transiently expressed an isolated kinase domain (JH1 domain) of Jak2-WT and mutants (Fig. 2C). An eYFP tag was added for detection purposes. Again, the ΔJSI, F1061A, M1064, and I1065A mutants showed no or reduced phosphorylation, whereas the M1062A mutation led to increased autophosphorylation, indicating that the observed level of phosphorylation is neither due to a regulatory interaction with the JH2 domain nor with the FERM or SH2 domains.
Expression of Jak2-M1062A leads to constitutive STAT activation

To further characterize the JSI mutants, we generated stable inducible transfectants expressing Jak2-WT or one of the four point mutants using the Jak2-deficient H9253 fibrosarcoma cells in which we inserted a FRT site. The different mutants were then inserted into the FRT site. This system thus avoids clonal differences because of the targeted transgene incorporation. Induction by doxycycline enables the expression of controlled levels of Jaks dependent on the doxycycline concentration used. Before the insertion of the Jak2 constructs, we stably transfected the cells with an EpoR construct. After induction of Jak2 expression with doxycycline, we investigated the phosphorylation of Jak2, STAT3, and STAT5. As depicted in Fig. 3, expression of the M1062A mutant leads to Jak2 autophosphorylation as well as to the phosphorylation of STAT3 and STAT5, whereas WT Jak2 did not elicit constitutive signaling. Furthermore, none of the other mutants induced signaling events.

JSI mutants display different cellular localization

Next, we generated HEK cells that stably express Jak2 proteins under the control of a doxycycline-inducible promoter. All proteins were N-terminally tagged with eYFP to monitor their localization in living cells using confocal laser scanning microscopy. To test the YFP-taggedJak2 construct, we stably and inducibly expressed YFP-Jak2 WT or a Jak2-FERM domain mutant (L87A/M88A) designed to abrogate receptor interaction in Jak2-deficient H9253-FRT cells and HEK-FRT cells. As shown in Fig. 4A, the YFP-tagged Jak2 protein is activated in a manner comparable to that of nontagged Jak2. Also, as described previously (21), YFP-Jak2-WT is localized predominantly at the plasma membrane with no detectable intracellular signal.
a slight cytoplasmic staining and an exclusion of Jak2 from the nucleus (Fig. 4B). In contrast, Jak2-L87A/M88A is not activated (Fig. 4A) and displays cytoplasmic localization (Fig. 4B).

We then evaluated the localization of the JSI mutants (Fig. 4C) in stable HEK-FRT cells using live cell microscopy. The inducibility of Jak2 expression is shown in lanes 1 and 3 (Fig. 4C). Membrane staining with trypan blue was provided in lanes 2, 4, and 6 (Fig. 4C). As demonstrated in lane 5 (Fig. 4C), the mutants M1062A and M1064A showed the same distribution as Jak2-WT. The mutant I1065A also displayed membrane localization but showed an increased portion of cytoplasmic protein. The effect of the F1061A mutant was even more pronounced, as YFP-Jak2-F1061A clearly displayed cytoplasmic localization. Colocalization with the membrane staining was hardly observed.

**Mutations in the JSI region can differentially affect the signaling of IFN-γ and erythropoietin**

To investigate whether mutations in the JSI region affect cytokine responses, we investigated signaling in response to IFN-γ and Epo in reconstituted γ2A-FRT cells expressing the different JSI mutants. Fig. 5 illustrates the signal transduction after stimulation of the cells with IFN-γ. First, we investigated the phosphorylation of Jak2 and Jak1. For this, Jak2 expression was induced with 1 μg/ml doxycycline (a dose for which M1062A shows little constitutive signaling) and the cells were stimulated with IFN-γ. After immunoprecipitation of Jak2 (Fig. 5A) or Jak1 (Fig. 5B), the phosphorylation status of the kinase was monitored. As shown in Fig. 5A, phosphorylation of Jak2 increased in cells expressing Jak2-WT, -M1062A, -M1064A, and -I1065A. However, in cells expressing the F1061A mutant, Jak2 phosphorylation was significantly reduced and cells expressing the F1061A mutant did not show Jak2 phosphorylation. These two mutants also affect the basal phosphorylation of Jak2 (Figs. 5A and 6A). In contrast, Jak1 phosphorylation was comparable in Jak2-WT, -M1062A, -M1064A, and -I1065A cells, whereas it was impaired in cells expressing the F1061A mutant (Fig. 5B). To investigate downstream signaling events, we next investigated the kinetics of STAT1 phosphorylation (Fig. 5C) as well as its dose-dependent activation (Fig. 5D) upon IFN-γ treatment. We observed that STAT1 phosphorylation was comparable in cells expressing Jak2-WT, -M1061A, -M1064A, and -I1065A.
but significantly reduced in cells expressing the F1061A mutant. A similar effect was visible when we checked STAT1 target genes that are up-regulated in response to IFN-γ/H9253. Only the F1061A mutant showed a clearly impaired induction of IRF1, GBP2, and STAT1 protein expression. It must be noted that a slight reduction in IRF1 and GBP2 expression was observed in cells expressing the I1065A mutant. This was also observed in a reporter gene experiment using an IRF1 promoter reporter construct (Fig. 1F). Reporter gene analysis consistently showed an induction of the reporter in nonstimulated cells expressing the M1062A mutant. This indicates that the M1062A mutant induces constitutive signaling that is not detectable in the Western blot analysis but becomes apparent in a reporter gene analysis integrating the STAT signals over a long time period.

Next, we studied signal transduction in the stably reconstituted H92532A cells upon treatment with Epo (Fig. 6). Again, we first checked the phosphorylation status of immunoprecipitated Jak2 upon Epo stimulation (Fig. 6A). Although Jak2 phosphorylation upon treatment with Epo is more pronounced in comparison to IFN-γ stimulation, the phosphorylation pattern for the different mutants is comparable with a clear reduction of Jak2 phosphorylation being observed for the I1065A mutant and a loss of phosphorylation occurring in cells expressing the F1061A mutant. As illustrated in Fig. 6B, this translates to downstream STAT5 activation. Whereas cells expressing Jak2-WT, -M1062A, or -M1064A display comparable phosphorylation of STAT5, the I1065A mutation significantly impairs Epo-mediated activation of STAT5. In Jak2-F1061A cells, STAT5 signaling upon Epo stimulation is abrogated. To further investigate this phenomenon, we performed a reporter gene analysis using a STAT5-dependent CIS promoter-reporter construct (Fig. 6C). Again, whereas the reporter gene induction in cells reconstituted with the WT protein and the two Met to Ala mutants is comparable, expression of I1065A and F1061A dramatically impairs the induction of this STAT5-dependent reporter. Of note, a constitutive induction of reporter gene activity was again apparent in nonstimulated cells expressing the M1062A mutant. Finally, we tested whether the different mutants are able to bind to the Epo receptor. Fig. 6D shows that all constructs can be coprecipitated with the EpoR upon induction of the protein with doxycycline. Jak2-F1061A, however, shows reduced binding to the EpoR.

**JSI mutants are susceptible to negative regulation via SOCS proteins**

As the SOCS proteins SOCS1 and SOCS3 are able to inhibit Jak2 kinase activity by directly binding to its kinase domain via their kinase inhibitory region and/or SH2 domain (25–27), we investigated whether JSI mutants could be inhibited by SOCS1 and SOCS3. To address this point, we expressed increasing

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**FIGURE 5.** IFN-γ signaling in cells stably expressing Jak2-WT or JSI mutants. A and B, Jak2-deficient γ2A-EpoR cells stably reconstituted with Jak2-WT or the different mutants were treated with 1 μg/ml doxycycline for 24 h to induce Jak2 expression and then stimulated with IFN-γ (10 ng/ml) for 15 min. Jak2 (A) or Jak1 (B) were precipitated and their phosphorylation was assessed by Western blot analysis. IP, Immunoprecipitation; pY, phosphotyrosine. C, Reconstituted γ2A-EpoR-Jak2 cells were treated with IFN-γ (10 ng/ml) for the times indicated. STAT1 activation and up-regulation was monitored by Western blot analysis. Detection with a Fin13 Ab is provided as loading control. D, γ2A-EpoR-Jak2-cells were treated with the indicated amounts of IFN-γ for 1 h and STAT1 activation was monitored. E, γ2A-EpoR-Jak2-cells were treated with 10 ng/ml IFN-γ for the times indicated and the expression of IRF1, GBP2, STAT1, and Fin13 (loading control) was monitored. F, γ2A-EpoR-Jak2-cells were transfected with a STAT1-dependent IRF1 promoter-reporter construct and Jak2 expression was induced with 1 μg/ml doxycycline. After stimulation of the cells with 10 ng/ml IFN-γ for 24 h, reporter gene activity was measured as described in Materials and Methods. Error bars represent the SD of a triplicate experiment.
Jak2 was precipitated and its phosphorylation was assessed by Western blot analysis. IP, Immunoprecipitation; pY, phosphotyrosine.

Important residue Phe1061 to alanine leads to the total loss of auto-

Discussion

In this study, we investigated the role of an unusual insertion, the Jak-specific insertion or JSI, in the kinase domain of Jak2. This insertion of ~15 aa, which is present in all Jak family members with the exception of the Drosophila homolog, encompasses an α-helical structure. This is lining the substrate-binding site of the kinase and lies in close proximity to the catalytic cleft of the enzyme (14). The location of this unusual insertion indicates its potential importance for kinase activity.

We performed a mutagenesis study for which we generated a JSI-deletion mutant as well as four point mutants of conserved hydrophobic residues. Our results clearly show that the JSI region is crucial for Jak2 activity. The mutation of the structurally important residue Phe1061 to alanine leads to the total loss of auto-

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amounts of SOCS1 (Fig. 7A) or SOCS3 (Fig. 7B) in reconstituted γ2A-FRT-EpoR cells expressing the different JSI mutants. Because both SOCS proteins also inhibit Jak1 activity, the specific effect of SOCS1 and SOCS3 on the Jak2 mutants was studied after stimulation with Epo using the STAT5-dependent CIS reporter construct. As apparent from Fig. 7, both SOCS1 and SOCS3 dose-dependently inhibit STAT5-dependent signaling in cells expressing the M1062A, M1064A, and I1065A mutants in a manner that is comparable to the inhibition of the WT Jak2 protein. As cells expressing the F1061A mutant do not display STAT5 activation, the regulation of this mutant could not be investigated. Because all JSI mutants that elicit STAT5 activation are sensitive to negative regulation by SOCS1 and SOCS3, it can be concluded that the JSI region is not involved in the negative regulation of Jak2 by these proteins.

As the activity of the kinase domain of Jaks can be modulated by other domains such as the kinase-like (JH2) domain or even the FERM domain (19, 28), we investigated whether the phosphorylation pattern we observed could be due to interactions with other Jak domains. Expressing an isolated kinase domain of Jak2-WT

FIGURE 6. Epo signaling in cells stably expressing Jak2-WT or JSI-mutants. A, Jak2-deficient γ2A-EpoR cells stably reconstituted with Jak2-WT or the different mutants were treated with 1 μg/ml doxycycline for 24 h to induce Jak2 expression and were then stimulated with Epo (10 ng/ml) for 15 min. Jak2 was precipitated and its phosphorylation was assessed by Western blot analysis. IP, Immunoprecipitation; pY, phosphotyrosine. B, γ2A-EpoR--Jak2cells were treated with the indicated amount of Epo for 1 h and STAT5 activation was monitored using a phospho-specific Ab, and Jak2, STAT5, and Fin13 (loading control) expression was assessed using the respective Abs. pY, Phosphotyrosine. C, γ2A-EpoR--Jak2 cells were transfected with a STAT5-dependent CIS promoter-reporter construct and Jak2 expression was induced with 1 μg/ml doxycycline. After stimulation of the cells with 10 ng/ml Epo for 24 h, reporter gene activity was measured as described in Materials and Methods. Error bars represent the SD of a triplicate experiment. D, γ2A-EpoR--Jak2-cells were treated with 1 μg/ml doxycycline (Dox) for 24 h to induce the expression of the different Jak2 proteins or were left untreated. After lysis of the cells, hemagglutinin (HA)-EpoR was precipitated and coprecipitated Jak2 was analyzed by Western blotting. IP, Immunoprecipitation.
and Jak2-mutants (Fig. 2C), we observed the same phosphorylation patterns as for the transiently expressed full-length proteins (Fig. 2B). Compared with Jak2-WT, the M1062A mutant displayed hyperphosphorylation and the other mutants showed reduced or no phosphorylation. Thus, the observed phosphorylation pattern and especially the observed hyperphosphorylation of the M1062A mutant are not due to impaired interactions with other domains of the kinase.

Next, we tested whether the point mutants were still sensitive to negative regulation by SOCS proteins. Both SOCS1 and SOCS3 directly bind to the Jak2 kinase domain (25, 26). As it was proposed that the kinase inhibitory region binds as a pseudosubstrate to the kinase domain of Jak2 (25), it is conceivable that the JSI region could be involved in this interaction. We therefore tested the dose-dependent inhibition of Epo-mediated signaling by SOCS1 and SOCS3 in Jak2-deficient cells reconstituted with WT Jak2 or the different mutants. We found the mutants M1062A, M1064A, and I1065A to be still sensitive to SOCS-mediated negative regulation (Fig. 7). The effect on the F1061A mutant could not be investigated, because Epo signaling is abrogated in cells expressing this mutant. However, as Phe\(^{1061}\) is a structural residue that is entirely buried within the structure, it cannot be involved in surface contacts with the SOCS proteins. Taken together, these data suggest that the JSI region is not involved in the negative regulation of Jak2 by SOCS1 and SOCS3.

Finally, we found that mutations in the JSI can differentially affect IFN-\(\gamma\) and Epo signal transduction. In Jak2-deficient cells reconstituted with different Jak2 constructs, we found that the Jak2 mutants M1062A, M1064A, and I1065A induce STAT1 phosphorylation to the same extent as WT Jak2 after IFN-\(\gamma\) stimulation (Fig. 5, C and D). Similarly, up-regulation of the STAT1-dependent proteins IRF1, GBP2, and STAT1 was also unaffected by these mutations (Fig. 5E). However, the F1061A mutant displayed impaired STAT1 signaling and reduced STAT1-dependent gene expression upon treatment with IFN-\(\gamma\). In clear contrast, the mutant I1065A showed a strongly decreased STAT5 activation (Fig. 6B) and STAT5-dependent CIS induction (Fig. 6C) after Epo stimulation. The signaling observed in cells expressing the M1062A and M1064A mutants was comparable to that of the signals initiated by WT Jak2 but was totally abrogated in F1061A-expressing cells. These data indicate that the JSI mutants behave differently depending on the triggered receptor system. In a homodimeric receptor complex involving two Jak2 molecules, the I1065A mutant is hardly able to induce downstream signaling, whereas it can elicit signaling in the context of a heterodimeric receptor complex such as the IFN-\(\gamma\) receptor recruiting different Jak5, namely Jak1 and Jak2. Similarly, although Jak2-F1061A can to some extent mediate signaling upon IFN-\(\gamma\) stimulation, it cannot initiate Jak2/STAT5 signaling after Epo stimulation. In the context of Epo stimulation, the signaling behavior of the mutants reflects the phosphorylation pattern of Jak2 (Fig. 6A). After treatment with IFN-\(\gamma\), however, the signaling characteristics reflect the phosphorylation pattern of Jak1 (Fig. 5B). The reduced Jak2 phosphorylation seen for I1065A, as well as the absence of phosphorylated Jak2 in the case of F1061A, nevertheless led to the activation of STAT1 and the induction of STAT1 target genes (Fig. 5, C–F). This does, however, not imply that Jak2 catalytic activity is not required for IFN-\(\gamma\) signaling, as STAT1 activation in \(\gamma2A\) cells reconstituted with a kinase-dead Jak2 mutant (Jak2-K882E) is abrogated (19, 29). Thus, although phosphorylation of the F1061A mutant cannot be detected, it must retain some catalytic activity. Taken together, the data show that mutations in Jak2, and in particular in the Jak2 kinase domain, may affect signaling via homomeric receptor complexes more dramatically than signals mediated via heteromeric receptors involving different Jak family members. It also suggests that the binding and/or activation mechanisms of Jak5 significantly differ in these settings. Interestingly, it was proposed that the presence of homodimeric class I cytokine receptors is needed for Jak2-V617F-mediated transformation (30). This mutant Jak2 protein carrying an activating mutation within the kinase-like domain is found in the majority of patients suffering from myeloproliferative diseases (5, 6). Overall, the intramolecular interactions between the different domains of Jak5, but also the contacts between two Jak molecules in receptor dimers, may account for significant differences.
in the activation mechanism of Jaks in the various cytokine receptor contexts.

Disclosures

The authors have no financial conflict of interest.

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