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To cite this version:
M. F. Richter, G. Duménil, G. Uze, M. Fellous, S. Pellegrini. Specific contribution of Tyk2 JH regions to the binding and the expression of the interferon alpha/beta receptor component IFNAR1. Journal of Biological Chemistry, American Society for Biochemistry and Molecular Biology, 1998, 273 (38), pp.24723–24729. 10.1074/jbc.273.38.24723. hal-02196867

HAL Id: hal-02196867
https://hal.archives-ouvertes.fr/hal-02196867
Submitted on 27 May 2021

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Cytokine signaling involves the activation of the Janus kinase (JAK) family of tyrosine kinases. These enzymes are physically associated with cytokine receptor components. Here, we sought to define the molecular basis of the interaction between Tyk2 and IFNAR1, a component of the interferon α/β receptor, by delimiting a minimal IFNAR1 binding region in the Tyk2 protein. Using an in vitro assay system, we narrowed down the interaction domain to a region comprising the JH7 and part of the JH6 homology boxes (amino acids 22–221). When expressed in Tyk2-negative cells, the JH7-6 region was unable to stabilize IFNAR1 protein levels, a critical function that we previously attributed to the N region (amino acids 1–591) of Tyk2. Moreover, substitution of the JH7-JH6 domain in JAK1 with that of Tyk2 did not restore IFNAR1 level nor interfere α signaling in Tyk2-negative cells. Thus, the major interaction surface lies within JH7-6, but additional JH regions (JH5-4-3) contribute in a specific manner to the in vivo assembly of Tyk2 and IFNAR1. Evidence is provided of the lack of specificity of the Tyk2 kinase-like and tyrosine kinase domains in interferon α/β receptor signaling.

The Janus kinase (JAK) family of non-receptor tyrosine kinases consists of four mammalian proteins (Tyk2, JAK1, JAK2, and JAK3) that play a critical role in initiating signaling cascades of a large number of cytokine receptors (1, 2). All JAK proteins possess a carbonyl-terminal tyrosine kinase (TK) catalytic domain, a central kinase-like (KL) domain, and a large amino-terminal (N) region, which has been subdivided into five JAK homology regions (JH7 to JH3) based on sequence conservation (3). The specific and noncovalent association of these kinases to the intracellular region of cytokine receptors governs their activation upon ligand binding (2). We are interested in understanding the mode of action and specific roles of Tyk2, which is activated, together with another JAK family member, by the type I interferons (IFN) (several α and one β subtypes), by interleukin (IL) 6, IL-10, and IL-12 (4–9).

The IFN-α/β receptor is present at low numbers on the surface of all cell types and consists of two transmembrane proteins called IFNAR1 and IFNAR2 (10, 11). The IFNAR2 gene generates several alternatively spliced forms, but only the product harboring a long intracytoplasmic domain (IFNAR2c) is part of a functional IFN-α/β receptor (12). Whereas the stoichiometry and spatial organization of these components within the receptor complex are unknown, the epitopes on the IFN molecule contacting IFNAR1 and IFNAR2 are being identified (13). High affinity binding of IFN-α/β to the receptor results in tyrosine phosphorylation and enzymatic activation of the associated JAK1 and Tyk2 in a defined temporal order, which is thought to result from the topology of each kinase within the complex (14, 15). Studies of kinase-deficient mutant cell lines showed that in the absence of either kinase, high affinity IFN-α binding is impaired, demonstrating a structural role of these enzymes in the formation of functional receptors (4, 16, 17). Our recent in vivo studies of deleted forms of Tyk2 expressed in Tyk2-deficient 11,1 cells have highlighted distinct functions of the protein toward the expression and the binding activity of the receptor complex. Each function appears to be contributed by a different domain adding more complexity to the receptor-kinase complex. The N region, previously defined as the amino-terminal 591 residues and comprising the JH7 to JH3 regions (Fig. 2), maintains the steady-state level of the IFNAR1 protein in the cell. The kinase-like domain contributes to the formation of high affinity receptor binding sites, and the tyrosine kinase domain is essential for optimal binding and signaling function (14, 18, 19).

That Tyk2 interacts physically with IFNAR1 was suggested by co-immunoprecipitation of the two endogenous proteins from cell extracts and by retention of baculovirus-expressed Tyk2 by a fusion protein bearing the cytoplasmic domain of IFNAR1 (20, 21). It was shown that the 45 membrane-proximal amino acids of IFNAR1 were necessary and sufficient for this interaction and that Ala substitution of three critical residues (Ile-Ile-Glu) disrupted this interaction (22). Because in vitro co-immunoprecipitation studies in reconstituted Tyk2-negative cells are hampered by the modulation of IFNAR1 level by Tyk2 itself (19), an in vitro system was used here to identify the molecular determinant(s) of Tyk2 which govern its interaction with IFNAR1. Using carboxyl- and amino-terminal deletion mutants of N, we delimited a region including JH7 and part of JH6 that is sufficient to bind recombinant IFNAR1. However, this region alone could not sustain IFNAR1 in Tyk2-negative cells, showing a requirement for additional JH boxes (JH5-4-3).
Thus, to address the specificity, if any, of this additional region, a number of Tyk2/JAK1 chimeric constructs were generated. Of four chimeras bearing different JH domains of Tyk2 fused to JAK1, only one could rescue IFNAR1 protein levels and IFN-α signaling. This functional chimera contains the entire N region of Tyk2 fused to the KL and TK domains of JAK1. These results will be discussed in the light of recent reports on other JAK/receptor pairs.

**MATERIALS AND METHODS**

**Plasmids**

Histidine-tagged Constructs—All constructs were made in pQE-based expression vectors (Qiagen). To generate plasmid pHis-N(1–451), an SphI fragment was released from plasmid bs-Tyk2 (human Tyk2 cDNA in pBlueScript) and cloned into pQEX10. pHis-N(1–451) encodes p53 (Fig. 2) containing the amino-terminal sequence Met-Arg-Gly-Ser-His-Thr-Asp-Pro-Arg-fused to Tyk2 amino acids 1–451 and the carboxy-terminal Arg-Arg-Pro-Ala-Ala-Lys-Leu-Asn sequence. pHis-N(1–591) was obtained by inserting a 140-base pair SacI/EcoRI fragment spanning amino acids 452–591 into the SacII-HindIII blunt-ended pHis-N(1–451) DNA. pHis-N(1–591) encodes p69 (Fig. 2) with the (His)6 sequence (see above) fused to Tyk2 amino acids 1–591 and the carboxy-terminal Ile-Glu-Leu-Ala-Sequences: pHis-N(1–591), pHis-N(1–385), pHis-N(1–369), pHis-N(1–314), and pHis-N(1–220) were generated by digesting plasmid pHis-N(1–451) with PstI and either PvuII, SacII, DraIII, or Nael, blunting the ends and re-circularizing each product. Due to the cloning procedure, the resulting proteins contain between 3 and 5 extra carboxy-terminal amino acids. The three amino-terminal deletion mutants were derived from pHis-N(1–385) (p46 in Fig. 2). A fragment encoding amino acids 22–385 of Tyk2 was amplified by PCR using primers with appropriate restriction sites and was ligated into BamHI/SphI-digested pHis-N(1–385) to generate pHis-N(22–385). The resulting protein (Δ21 in Fig. 2) contains the amino-terminal His sequence (see above) fused to Tyk2 amino acids 22–385 and to Ala-Lys-Leu-Asn. pHis-N(1–385) was digested with Stau and HindIII, the resulting 1.1-kb fragment was ligated to the HindII-HindIII-digested pQEX10 vector to generate pHis-N(25–385). The resulting protein (Δ27 in Fig. 2) contains the amino-terminal His sequence fused to Tyk2 amino acids 28–385 of Tyk2 and Ala-Lys-Leu-Asn. pHis-N(1–385) was digested with PvuII and HindIII, and the resulting 1-kb fragment was ligated to the HindII-HindIII-digested pQE11 vector to generate pHis-N(54–385). The protein (Δ53 in Fig. 2) contains the amino-terminal His sequence fused to Tyk2 amino acids 54–385 and Ala-Lys-Leu-Asn.

**GST Fusion Constructs**—The cytoplasmic domain of the human IFNAR1 (amino acids 458 to 557) cloned into the bacterial pGEX-2T expression vector was provided by L. Ling (Biogen Inc., Boston, MA). The Ile-Ile-Glu→Ala mutant (amino acids 504–506) was obtained by two-step PCR-mediated mutagenesis. The GST-IkB receptor pairs.

**Interaction between Tyk2 and IFNAR1**

Histidine-tagged proteins were expressed in bacteria, purified on nickel-nitrotriacetic acid (Ni²⁺-NTA) agarose beads according to the manufacturer’s protocol (Qiagen), eluted, and dialyzed against 50 mM Tris-HCl, pH 8, 100 mM KCl, 5 mM MgCl₂, 20% glycerol, and 0.1% Nonidet P-40. GST fusion proteins were affinity-purified on glutathione-Sepharose (Amersham Pharmacia Biotech), eluted with 50 mM Tris-HCl, pH 8, 10% glycerol, 10 mM glutathione, and stored at −80 °C. Approximately 10 to 100 pmol of each freshly purified recombinant protein was incubated in 1× phosphate-buffered saline for 60 min at 4 °C in a total volume of 1 ml. Fifty μl of glutathione-Sepharose beads as a 50% slurry were added. Beads were pelleted and washed four times in 1× phosphate-buffered saline, 0.5 mM NaCl. Bound proteins were boyled in Laemmli sample buffer, separated by SDS-PAGE, and analyzed by Coomassie staining or immunoblotting with the appropriate mAbs (anti-N, anti-IgG) and anti-MxA (gift of R. Weil). The GST mAb used in Western blot was kindly provided by Hybri-dolab (Institut Pasteur). The histidine-tag mAb was purchased (Di-anova). Tyk2 antibodies used for immunoprecipitation and Western blotting (R5 and T10–2) have been described (19). Anti-IFNAR1 mAb IA12 and GB8 were from Biogen Inc. The anti-phosphotyrosine 4G10 antibody (Amersham Pharmacia Biotech) was a kind gift from Z. Reichmann. The anti-human IFN-α2b antibody (kindly provided by D. Gewirtz, Wellcome) was purchased from Sigma. Preparation of cell extracts, immunoprecipitations, and Western blot analysis were performed as described previously (14).

**RESULTS**

In Vitro Interaction of N and IFNAR1cyt—In vitro interaction assay was set up to identify an IFNAR1 binding domain within the first 591 amino acids (the N region) of Tyk2. Histidine-tagged N was expressed in *Escherichia coli* and purified as a 69-kDa protein (p69) on Ni²⁺-NTA agarose beads. The cytoplasmic domain of IFNAR1 was expressed as GST fusion protein (GST-IFNAR1cyt) and purified by glutathione-Sepharose affinity chromatography. The two proteins were co-incubated, and the complex retained on Ni²⁺-NTA-agarose beads was analyzed by SDS-PAGE and Coomassie staining. GST-IFNAR1cyt was retained on beads in the presence, but not in the absence, of p69 (Fig. 1A, lanes 2 and 3).

In subsequent experiments, proteins retained on beads were analyzed by Western blotting with the Tyk2 or the GST mAbs. In Fig. 1B, p69 was incubated with either the GST-IFNAR1cyt protein, a mutated version of it in which residues 504 to 506 (Ile-Ile-Glu) were replaced by alanines (22), or with a control fusion protein (GST-IkBβ). The material retained on glutathione-Sepharose beads was analyzed with Tyk2 mAb p69 bound to GST-IFNAR1cyt and to a much lesser extent to the mutated version, albeit it did not bind to GST-IkBβ (Fig. 1B, lanes 1–3). To confirm this interaction, the reverse experiment was performed, where Ni²⁺-NTA-agarose beads were used to pull down the complex and the GST mAb was used for blotting; GST-IFNAR1cyt was able to interact with p69, but not with a control His-MxA protein (Fig. 1C). Taken together, these results show and the VSV-G epitope at the 3′ end. After sequencing (370A DNA Sequencer, Applied Biosystems), all resulting cDNAs were introduced into the pRc/CMV vector.

**Cell Culture**

The mutant cell line 11,1 (also called U1A) has been described (16). Cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal calf serum and 250 μg/ml hygromycin. Calcium phosphate/DNA transfection and selection in G418 (450 μg/ml) was performed as described (18). Cell survival in hypoxanthine/aminopterin/thymidine (HAT) or in 6-thioguanine (6TG) containing media was assayed in the presence of different concentrations of IFN-α2 (human recombinant IFN-α2b, kindly provided by D. Gewirtz, Wellcome). Binding experiments with 125I-IFN-α2c were performed as described (19).

**Antibodies, Immunoprecipitation, and Immunoblotting**

The GST mAb used in Western blot was kindly provided by Hybri-dolab (Institut Pasteur). The histidine-tag mAb was purchased (Di-anova). Tyk2 antibodies used for immunoprecipitation and Western blotting (R5 and T10–2) have been described (19). Anti-IFNAR1 mAb IA12 and GB8 were from Biogen Inc. The anti-phosphotyrosine 4G10 antibody (Amersham Pharmacia Biotech) was a kind gift of Z. Reichmann. The anti-human IFN-α2b antibody (kindly provided by D. Gewirtz, Wellcome) was purchased from Sigma. Preparation of cell extracts, immunoprecipitations, and Western blot analysis were performed as described previously (14).
that the N region of Tyk2 interacts specifically with the cytoplasmic domain of IFNAR1 in vitro. This interaction is impaired by alanine substitution of three IFNAR1 residues that were previously shown to be critical for optimal Tyk2 binding (22).

The JH7-6 Region of N Interacts with IFNAR1cyt in Vitro—To initially delimit an IFNAR1 binding domain within N, five carboxyl-terminal truncated versions of His-N were generated and named according to their apparent molecular weights (Fig. 2). Each protein was tested for its ability to bind GST-IFNAR1cyt (Fig. 3). All five carboxyl-terminal deleted versions of p69 retained the ability to interact with GST-IFNAR1cyt (Fig. 3, lanes 1, 3, 5, 7, 9, and 11), whereas they did not interact with the control GST-IκBβ fusion protein (Fig. 3, lanes 2, 4, 6, 8, 10, and 12). This result was confirmed upon using the Ni²⁺-NTA resin-based assay (Fig. 4A). Thus, the amino-terminal 221 residues of N, comprising JH7 and part of JH6, retain the ability to interact with IFNAR1cyt.

To delimit the amino-terminal boundary of this binding domain, three amino-terminal deletions were generated from the p46 protein, lacking 21, 27, and 53 amino acids, respectively (Δ21, Δ27, and Δ53 in Fig. 2). The purified proteins were tested for their ability to interact with wild-type or the mutant GST-IFNAR1cyt in pull-down experiments using either of the two resins. Δ21 did not interact with GST-IFNAR1cyt (Fig. 4A, lanes 5 and 7 and Fig. 4B, lane 2), whereas, as shown above, p46 and p27 bound specifically to wild-type GST-IFNAR1cyt (Fig. 4A, lanes 1 and 3). On the other hand, Δ21 fusion protein retained IFNAR1 binding capacity (Fig. 4B, lane 1). These results demonstrate that the first 21 amino acids are dispensable for the in vitro binding of N to IFNAR1 and that the binding domain boundary is situated between residues 22 and 28.
The recombinant p27 protein, lacking JH5-4-3, was expressed in Tyk2-negative cells a mutant form derived from the full-length Tyk2 and lacking amino acids 1–21 (Δ1–21). Two independent neo<sup>+</sup> transfectants were studied. As a measure of in vivo Tyk2 function, we first analyzed the levels of endogenous IFNAR1. The Δ1–21 clones were compared with WT cells expressing wild-type Tyk2, to Δ1–51 cells expressing a Tyk2 deleted residues 1–51 (19), and to 11,1 cells. As can be seen in Fig. 5A, unlike Δ1–51, expression of Δ1–21 restored IFNAR1. In light of the different in vitro IFNAR1 interaction capacity of Δ21 and Δ53, this result is likely to reflect the different ability of the two deletion mutants to physically interact with IFNAR1 in vivo. We also measured the IFN-α binding activity of Δ1–21 expressing cells, using WT and 11,1 cells as controls. As shown in Fig. 5B, iodinated IFN-α bound similarly to WT and to Δ1–21 expressing cells, whereas it did not bind to 11,1 cells or to Δ1–51 expressing cells (see also Fig. 5 in Ref. 19). Furthermore, no difference in the sensitivity to IFN-α could be measured between the Δ1–21 clones and WT cells upon their phenotype in HAT or 6TG media (18) (data not shown). Thus, we conclude that the amino-terminal 21 residues of Tyk2 are dispensable not only for the in vitro binding of N to IFNAR1 but also for the in vivo structural and signaling functions of Tyk2 through the IFN-α receptor.

Tyk2 JH5-4-3 Are Specifically Required to Sustain IFNAR1—The recombinant p27 protein, lacking JH5-4-3, was as effective as full-length p69 in complexing with IFNAR1 (the indicated His-tagged proteins were incubated with the wild-type IFNAR1 or the mutated (IIE → AAA) version of GST-IFNAR1cyt. Proteins retained on nickel beads were analyzed by Western blotting with GST antibody). The indicated His-tagged proteins were incubated with GST-IFNAR1cyt. Proteins retained on glutathione-Sepharose were analyzed by Western blotting with the Tyk2 mAb.

Tyk2 Lacking Amino Acids 1–21 Is Able to Restore Signaling in 11,1 Cells—To correlate the in vitro binding results (Fig. 4) with the function of the native protein in cells, we stably expressed in Tyk2-negative cells a mutant form derived from the full-length Tyk2 and lacking amino acids 1–21 (Δ1–21). Two independent neo<sup>+</sup> transfectants were studied. As a measure of in vivo Tyk2 function, we first analyzed the levels of endogenous IFNAR1. The Δ1–21 clones were compared with WT cells expressing wild-type Tyk2, to Δ1–51 cells expressing a Tyk2 deleted residues 1–51 (19), and to 11,1 cells. As can be seen in Fig. 5A, unlike Δ1–51, expression of Δ1–21 restored IFNAR1. In light of the different in vitro IFNAR1 interaction capacity of Δ21 and Δ53, this result is likely to reflect the different ability of the two deletion mutants to physically interact with IFNAR1 in vivo. We also measured the IFN-α binding activity of Δ1–21 expressing cells, using WT and 11,1 cells as controls. As shown in Fig. 5B, iodinated IFN-α bound similarly to WT and to Δ1–21 expressing cells, whereas it did not bind to 11,1 cells or to Δ1–51 expressing cells (see also Fig. 5 in Ref. 19). Furthermore, no difference in the sensitivity to IFN-α could be measured between the Δ1–21 clones and WT cells upon their phenotype in HAT or 6TG media (18) (data not shown). Thus, we conclude that the amino-terminal 21 residues of Tyk2 are dispensable not only for the in vitro binding of N to IFNAR1 but also for the in vivo structural and signaling functions of Tyk2 through the IFN-α receptor.

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virus was also assayed. With WT cells and two clones expressing the T-(1–581)-J chimera, the dose range for antiviral protection was between 100 pM and 1 pM IFN-α. Conversely, with T-(1–518)-J and 11,1 cells no protection was observed with up to 100 nM IFN-α. Thus, a chimera bearing the N region of Tyk2 and the KL and TK domains of JAK1 can fully replace wild-type Tyk2 in the IFN-α pathway.

**DISCUSSION**

In this study, two approaches were used to delimit the minimal region within the tyrosine kinase Tyk2 that is required to interact with the IFNAR1 component of the IFN-α/β receptor complex. An in vitro approach for testing the interaction of the two recombinant partners narrowed down the IFNAR1cyt interaction domain to Tyk2 amino acids 1–221, spanning the homology region JH7 and part of JH6. The amino-terminal boundary of the domain was mapped between amino acids 21 and 27. We could not delimit more accurately the carboxy-terminal boundary of the domain because the expression level of a shorter construct (amino acids 1–172) dropped considerably. The physiological relevance of this in vitro analysis is supported by the finding that in 11,1 cells, a mutant form of Tyk2 lacking amino acids 1–21 was as functional as the wild-type protein, i.e. it restored IFNAR1 levels, ligand binding, induced gene expression, and antiviral protection. In contrast, further deletion to amino acid 51 abolished all functions. Alignment and close inspection of the amino-terminal end of the JAK family members revealed some interesting features (Fig. 8) that may explain the different properties of the deletion mutants. The first portion (20–25 residues) of this segment is variable in length, displays low identity, and lacks conserved secondary structures. Starting around Tyk2 position 28, however, approximately at the amino boundary of the JH7 region (3), predicted secondary structures common to the different family members can be found (Fig. 8). The result of our analysis of amino-terminal deletion mutants together with this prediction suggest that these structural elements are critical for the function and/or stability of the domain.

Recent studies of other JAK proteins have similarly suggested the existence of a JH7-6 structural element within the first 200 residues that would constitute a specific interaction surface for cytokine receptor chains (2, 23, 24). Functional analysis of JAK2 deletion mutants or of JAK2/JAK1 chimeras, expressed in JAK2-negative cells, showed that the first 251 residues of JAK2, spanning JH7-6, were specifically required for the interaction with the R2 subunit of the IFN-γ receptor (25). Studies of the interaction of the γc chain of the IL-2, -4, -7, -9, and -15 receptors with deleted forms of JAK3 expressed in COS cells narrowed down the domain of interaction to JH7-6 (JAK3 residues 1–192) as well (26). This JH7-6 region alone was not tested in a more physiological system, although the same authors did show that a chimera containing JH7-6-5 and part of JH4 of JAK3 (residues 1–370) fused with JAK2 could restore IL-2 signaling in JAK3-negative cells stably reconstituted with the αβγ components of the IL-2 receptor. On the other hand, structure-function analysis of JAK1 in the context of the IFN-γ receptor showed that the entire amino-terminal region (JH7-JH3), fused to the kinase domains of JAK2, was required for the interaction with the R1 subunit of the IFN-γ receptor in JAK1-negative cells (25). In this system, the level of

**Fig. 6. Level of IFNAR1 in 11,1 cells expressing Tyk2/JAK1 chimera.** A, diagram of four Tyk2/JAK1 chimeras. Numbers indicate Tyk2 residues. The carboxy-terminal black rectangle indicates the VSV-G epitope. B, 11,1 cells and neo clones expressing wild-type Tyk2 (WT), JAK1, or the indicated chimera were analyzed for the IFNAR1 level as described in Fig. 5. Lower panel, the level of expression of Tyk2 and of the two indicated chimeras was analyzed by direct anti-Tyk2 Western blot from 20 μg of crude extract.

**Fig. 7. A**, tyrosine phosphorylation of chimeras T-(1–518)-J and T-(1–581)-J. Anti-Tyk2 immunoprecipitates from lysates of cells untreated (−) or treated (+) for 15 min with 1000 IU/ml IFN-α were immunoblotted with antibodies to phosphotyrosine (upper panel) and reprobed with antibodies to Tyk2 (lower panel). Note that the molecular sizes of the two chimeras (125 and 132 kDa) approach that of Tyk2 (134 kDa). B, tyrosine phosphorylation of endogenous JAK1 in cells expressing Tyk2, chimera T-(1–518)-J, or chimera T-(1–581)-J. Lysates of cells untreated (−) or treated (+) for 15 min with 1000 IU/ml IFN-α were immunoprecipitated with JAK1 antibodies (M7) specific for the TK domain, blotted with antibodies to phosphotyrosine (upper panel), and reprobed with antibodies to JAK1 (lower panel).
the γ receptor protein was independent from the presence of the interacting JAK1. Thus, it appears that the requirements for JH regions other than JH7-6 vary in different receptor/JAK systems and this may relate to the variation in the affinities of the interaction between given partners.

In the present study, we show that, despite its ability to associate with IFNAR1 in vitro, the JH7-6 domain of Tyk2 is not sufficient to rescue IFNAR1 levels when tested in Tyk2-negative cells, indicating a critical requirement for the JH5-4-3 regions for this function. Moreover, our analysis of the Tyk2/JAK1 chimeras (Fig. 6) clearly showed that these regions from JAK1 could not functionally replace the Tyk2 ones, indicating strict specificity. These results suggest that JH5-3 is involved in the IFNAR1 stabilizing function. The mechanism by which this occurs is unknown (19), and no other examples of such phenomenon have been described to date. We cannot exclude the possibility that the JH5-3 region augments the affinity of the interaction of the JH7-6 binding domain with IFNAR1 or that it interacts with another component of the receptor complex. Whatever the mechanism, this contribution would be negligible in the in vitro system and become critical in the cellular environment. A study was recently reported on the IFNAR1 binding region of Tyk2 (27) which showed, in accordance with our in vivo data, that an intact N region was required for maximal binding to IFNAR1. This group also used lysates from bacteria expressing GST-Tyk2 fusions to pull down a CD4-IFNAR1 chimera transfected into 293T cells and proposed the existence of two weak binding sites centered around the JH6 and the JH3 boxes, respectively. We have no evidence of such weak interaction domains, and this discrepancy could be due to the different stringencies of the two experimental approaches. More sensitive methods will be required to define the relative contribution, if any, of independent JH boxes.

Our analysis of the chimeric Tyk2/JAK1 proteins also provides some clues about the specificity of the KL and the TK domains. Full restoration of function (gene induction and antiviral protection) by the T-(1–581)-J chimera demonstrates the interchangeability of these domains for Tyk2 function in cellular responses to IFN-α. Similar conclusions were reached for a JAK1/JAK2 chimera in the IFN-γ response (25). Although the function of the KL domain is not yet known, we have previously shown that, in Tyk2, it contributes to the ligand binding activity of the receptor (18, 19). Given the rescuing capacity of the T-(1–581)-J chimera, which contains the KL domain of JAK1, we conclude that this function of KL is not Tyk2-specific. IFN-α/β-induced activation of the JAKs is thought to involve trans-phosphorylation of regulatory tyrosine(s) located in the activation loop of the TK domain. This event takes place in a specific temporal order (JAK1 → Tyk2) with JAK1 playing the prominent role (14, 15). We previously proposed that this order may relate more to spatial constraints and stoichiometry of the receptor-kinase complex than to properties intrinsic to each kinase. Our present finding that endogenous JAK1 appears phosphorylated equally well when juxtaposed to the T-(1–581)-J chimera or to Tyk2 (Fig. 7B) provides further evidence of the lack of specificity of these kinases. To fully demonstrate this, it will be necessary to investigate whether the reciprocal chimera, bearing the α region from JAK1 fused to the kinase domains of Tyk2, can restore IFN-α responses in JAK1-negative cells. In this regard, it has been reported that a chimera bearing the α region of JAK1 fused to the kinase domains of Tyk2 can sustain substantial though incomplete IFN-α-induced gene expression and antiviral protection to EMC virus (25). Whether other biological responses to IFN-α, not measurable in our system, could specifically require Tyk2 kinase domains cannot be ruled out.

The analysis of the IFNAR1/Tyk2 pair points to a variant, i.e., the expression level and the intrinsic stability of endogenous cytokine receptor subunit, which could be critical for some receptor-kinase complexes and/or in some cell types. It will be interesting to investigate whether the Tyk2 domains defined here for the assembly with IFNAR1 overlap with those involved in the interaction with other Tyk2-activating cytokine receptors, such as the IL-12 or the IL-10 receptors (28, 29). Similarly, it remains to be seen whether any specificity in the function of the kinase domains of Tyk2 could be revealed through the study of these two other receptor-kinase complexes.

Acknowledgments—We thank M. C. Gauzzi, T. C. Yeh, and V. Di Bartolo for discussions and critical review of the manuscript; H. K. Lorenzo for valuable and generous help in collecting data and preparing Fig. 8; K. Siew-Lai and R. Schreiber for providing the sequence of the murine Tyk2 protein. We also thank A. Wilks for providing the human JAK1 cDNA, A. Ziemiecki for the JAK1 antibodies, L. Ling for the GST-IFNAR1 construct and the IFNAR1 antibodies, and R. Weil for the GST-IkBβ chain construct.

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