Interaction of the Transcriptional Activator Stat-2 with the Type I Interferon Receptor*

(Received for publication, July 11, 1995, and in revised form, August 25, 1995)

Shahab Uddin, Aghiad Chamdin, and Leonidas C. Platanias†

From the Division of Hematology-Oncology, Department of Medicine, Loyola University Chicago, Maywood, Illinois 60153 and Hines Veterans Administration Hospital, Hines, Illinois 60141

Binding of interferon-α (IFNα) to the multisubunit type I IFN receptor (IFNR) induces activation of the Tyk-2 and Jak-1 kinases and tyrosine phosphorylation of multiple signaling elements, including the Stat proteins that form the ISGF3x complex. Although Jak kinases are required for IFNα-dependent activation of Stats, the mechanisms by which Stats interact with these kinases are not known. We report that Stat-2 associates with these kinases within 1 min of interferon treatment of cells, and is inducible by various type I (α) IFNs. The kinetics of Stat-2-IFNR association are similar to the kinetics of phosphorylation of Stat-2, suggesting that during its binding to the type I IFNR, Stat-2 acts as a substrate for interferon-dependent tyrosine kinase activity. These findings support the hypothesis that the type I IFNR acts as an adaptor, linking Stat proteins to Jak kinases. Interaction of Stat-2 with the β2 subunit of the type I IFNR may be a critical signaling event, required for the formation of the ISGF3x complex and downstream transcription of interferon-stimulated genes.

In order for type I interferons to exert their pleiotropic biological effects on cells and tissues, binding to the type I IFN receptor (IFNR) is required (1). Previous studies have established that the type I IFNR has a multisubunit structure (2–6). In affinity cross-linking studies of 125I-IFNα to the type I IFNR, 125I-IFNα-2-IFNR complexes with approximate molecular masses of 130–140 kDa (α subunit), 110–120 kDa (β subunit), 210–230 kDa (that appears to result from an association of the α and β subunits), and less prominent complexes of 75 and 180 kDa (most likely an association of the α subunit with the 75-kDa complex) are detected (2–7). The variant of the type I IFNR, expressed in some myelomonocytic cell lines, is characterized by lack of expression of the 110–120- and 210-kDa complexes and the presence of 125I-IFNα-2-IFNR complexes of 130–140 kDa (α subunit), 75 kDa, and 180 kDa (association of the α subunit with the 75-kDa complex) (2, 6). The cloning of the genes encoding two subunits of the type I IFNR has been reported (8, 9). The subunit cloned by Uzé et al. (8) has been shown to correspond to the previously described α subunit of the receptor (10). The relative molecular mass of the α subunit appears to exhibit slight variations in different cell lines, ranging from 110 to 135 kDa (2, 6, 11, 12), possibly due to differential glycosylation of the protein (4). The subunit cloned by Novick et al. (9) has been reported to encode for a 51-kDa protein. Domanski et al. (7) have recently cloned a cDNA that encodes a 100-kDa form of the type I IFNR. This receptor form and the one cloned by Novick et al. (9) have identical extracellular and transmembrane domains and the first 15 amino acids of the cytoplasmic domain but differ in the rest of the cytoplasmic region (7). In the current study, we used antibodies generated against the receptor subunits cloned by Uzé et al. (8) and by Novick et al. (9) to further characterize the structure of the type I IFNR and its interactions with other signaling molecules. To avoid confusion in the terminology of the different subunits and to be consistent with the terminology used by other groups (7), we will refer to the product of the gene cloned by Uzé et al. (8) as the α subunit of the type I IFN receptor, the product of the gene cloned by Novick et al. (9) as the β2 subunit of the type I IFN receptor, and the product of the gene cloned by Domanski et al. (7) as the β1 subunit of the type I IFNR. Our findings demonstrate that during type I IFN stimulation, the transcriptional activator Stat-2 associates with the β2 subunit of the type I IFNR, providing direct evidence for an interaction of this member of the Stat family of proteins with a specific component of the type I IFNR.

**EXPERIMENTAL PROCEDURES**

Cells and Reagents—The U-266 (human multiple myeloma), Daudi (lymphoblastoid), and Molt-4 (acute T cell lymphocytic leukemia) cell lines were grown in RPMI 1640 (Life Technologies, Inc.) supplemented (lymphoblastoid), and Molt-4 (acute T cell lymphocytic leukemia) cell lines were grown in RPMI 1640 (Life Technologies, Inc.) supplemented
We performed experiments in which $^{125\text{I}}$-IFN bodies raised against the different type I IFN receptor subunit (5, 6) (Fig. 1) association of the high molecular weight complex (HMWC-1) migrating at approximately 180 kDa (HMWC-2) was also seen in total cell lysates and was weakly immunoprecipitated by both the IFNaRC-1 and IFNaRC-2 antibodies. Similar results were obtained when the Molt-4 human cell line was studied, except that the receptor complexes migrated slightly slower in these cells (approximately 15–20 kDa difference), a finding consistent with the reported variations in the mobility of type I IFN components in different cell lines (11). Taken altogether, the results of the affinity cross-linking experiments strongly suggested that two distinct forms of the type I IFN receptor are co-expressed on the surface of human cells. The form of the receptor immunoprecipitated by the IFNaRC-2 antibody is consistent with the previously described variant form of the type I IFNR (2, 6). Further studies are required, however, to characterize the exact interactions between different receptor subunits and to establish that the form precipitated by the IFNaRC-2 antibody corresponds to the previously described variant receptor form (2, 6).

We subsequently performed studies in which cells were treated with IFNa and cell lysates were immunoprecipitated with the anti-receptor antibodies, analyzed by SDS-PAGE, and immunoblotted with anti-phosphotyrosine. Fig. A and B, shows that the $\alpha$ subunit of the receptor is tyrosine-phosphorylated in response to IFNa treatment of cells, in agreement with our previous findings using a monoclonal antibody against this subunit (14, 15). In addition, the IFNaRC-1 antibody co-immunoprecipitated an interferon-dependent tyrosine-phosphorylated protein with an $M_r$ of 135 kDa, corresponding to the phosphorylated form of the tyrosine kinase Tyk-2 (16). Immunoblotting of anti-IFNaRC-1 immunoprecipitates with a monoclonal anti-Tyk-2 antibody demonstrated that Tyk-2 is associated with the $\alpha$ subunit of the type I IFNR prior to and after IFNa stimulation (Fig. 2C), confirming the findings of a previous study (16) that had established an association of the $\alpha$ subunit with Tyk-2. Tyk-2 was not detectable in immunoprecipitates obtained with the anti-IFNaRC-2 antibody (Fig. 2C), suggesting that this kinase does not associate with the $\beta$ subunit of the receptor. Fig. 3A shows an experiment in which cell lysates from IFNa-treated cells were immunoprecipitated with the IFNaRC-2 antibody and immunoblotted with anti-phosphotyrosine. A band corresponding to the 51-kDa $\beta$ subunit could not be detected in such immunoblots, perhaps because it co-migrates with the heavy chain of rabbit immunoglobulin. Also no bands migrating at 102 kDa that would correspond to a phosphorylated receptor dimer were detectable. A 113-kDa tyrosine-phosphorylated protein, however, was clearly co-immunoprecipitated by this antibody upon treatment of cells with IFNa. As the $M_r$ of this protein was identical to the $M_r$ of the transcriptional activator Stat-2, we sought to determine whether it corresponds to Stat-2. Fig. 3B shows an anti-Stat-2 immunoblot on immunoprecipitates obtained with the IFNaRC-1 or IFNaRC-2 antibodies. Stat-2 is not present in IFNaRC-1 immunoprecipitates, but it is clearly detectable in IFNaRC-2 immunoprecipitates from IFNa-treated cells. Thus, Stat-2 appears to specifically associate with the $\beta$ but not with the $\alpha$ subunit of the type I IFNR. The kinetics of the association of Stat-2 with the $\beta$ subunit were subsequently

**RESULTS AND DISCUSSION**

We initially sought to determine the specificity of the antibodies raised against the different type I IFN receptor subunits. We performed experiments in which $^{125\text{I}}$-IFNa2 was cross-linked to its receptor on U-266 cells, the cells were lysed, and cell lysates were either analyzed directly by SDS-PAGE (lane 1) or immunoprecipitated with IFNaRC-1 (lane 2) or IFNaRC-2 (lane 3) or preimmune rabbit serum (lane 4) prior to SDS-PAGE analysis. The gel was dried, and bands were visualized by autoradiography. A band at 110–120 kDa could be distinguished in lane 1 on shorter exposure of the autoradiogram (data not shown). B, Molt-4 cell lysates obtained after affinity cross-linking of $^{125\text{I}}$-IFNa2 to its receptor were immunoprecipitated with IFNaRC-1 (lane 1) or IFNaRC-2 (lane 2) or preimmune serum (lane 3) prior to SDS-PAGE analysis.
studied. Fig. 4 shows an experiment in which Daudi cells were treated for different times with IFN-α, and after cell lysis, the lysates were immunoprecipitated with IFN-αRC-2 antibody (lanes 1 and 2) or preimmune rabbit serum (lane 6) or an antibody against Stat-2 (lane 7) and immunoblotted with an αStat-2 antibody. A weak band corresponding to Stat-2 could be detected at 90 min in the IFN-αRC-2 immunoprecipitates (lane 6) after longer exposure of the same blot (data not shown).

We have previously shown that different type I IFNs induce tyrosine phosphorylation of a common set of signaling proteins, including the α and β (100 kDa) subunits of the type I IFNR (14, 15), the Tyk-2 and Jak-1 kinases (15), Stat-2 and Stat-1 (15), p95vav (17), and insulin receptor substrate (IRS) proteins (18).2 These data have suggested that all type I IFNs activate common signaling cascades. However, differences among the signaling pathways of different type I IFNs also exist, as suggested by our finding that IFN-β selectively phosphorylates p100, a protein that associates with the α subunit of the type I IFNR (15). To determine whether different IFNs induce an

2 L. C. Platanias, S. Uddin, A. Yetter, X-J. Sun, and M. F. White, manuscript in preparation.
Stat-2 Associates with the Type I IFN Receptor

Our findings also provide some hints on the kinase activity responsible for Stat-2 phosphorylation. Colamonici et al. (10, 16) have reported that the α subunit of the receptor forms a complex with the tyrosine kinase Tyk-2, a finding confirmed by us using the IFNα-RC-1 antibody. Novick et al. (9) used an antibody that apparently detects both forms of the β subunit (51 and 102 kDa) and were able to demonstrate an association with the tyrosine kinase Jak-1. As Stat-2 appears to interact specifically with the β, but not the α subunit of the receptor, it is tempting to hypothesize that Stat-2 acts as a specific substrate for Jak-1 but not Tyk-2. Furthermore, as the Stat-2–IFN association is IFNα-dependent, it is possible that it involves binding of the SH2 domain of Stat-2 to the β subunit of the type I IFN. Such a model for an interaction of Stat-2 with the type I IFN would be also consistent with the findings of a recent study that demonstrated that the SH2 domain of Stat-2 is the determinant of signaling specificity, while Tyk-2 is not specifically required for Stat-2 phosphorylation (23). It remains to be determined whether Stat-1 also utilizes components of the type I IFN for its interaction with Jak(s). Interestingly, a recent study has demonstrated that phosphorylation of Stat-2 is required for activation of Stat-1, but not vice versa, suggesting that one binding site necessary for activation of Stat-1 may be the phosphotyrosine of Stat-2 itself (24). Taken together with our data, these findings raise the possibility that binding of Stat-2 to the β subunit of the type I IFN is the critical event required for the formation of the ISGF3α complex and downstream transcription of ISGs.

Acknowledgments—We thank Dr. Oscar R. Colamonici for sharing with us data from his laboratory prior to publication.

REFERENCES
1. Petska, S., Langer, J. A., Zoon, K. C., and Samuel, C. E. (1987) Annu. Rev. Biochem. 56, 727–777
2. Colamonici, O. R., Pfeffer, L. M., D’Alessandro, F., Platanias, L. C., Gregory, S. A., Rosden, A., Nordan, R., Cruciani, R. A., and Diaz, M. O. (1992) J. Immunol. 148, 2126–2132
3. Colamonici, O. R., D’Alessandro, F., Diaz, M. O., Gregory, S. A., Neckers, L. M., and Nordan, R. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7230–7234
4. Platanias, L. C., Pfeffer, L. M., Cruciani, R., and Colamonici, O. R. (1993) J. Immunol. 150, 3382–3388
5. Colamonici, O. R., and Domanski, P. (1993) J. Biol. Chem. 268, 10895–10899
6. Colamonici, O. R., Domanski, P., Królewski, J. J., Fu, X-Y., Reich, N. C., Pfeffer, L. M., Sweet, M. E., and Platanias, L. C. (1994) J. Biol. Chem. 269, 5660–5665
7. Domanski, P., Witte, M. M., Kellum, M., Rubinstein, M., Hackett, R., Pitha, P., and Colamonici, O. R. (1995) J. Biol. Chem. 270, 21606–21611
8. Uezu, G., Lutfiafla, G., and Greaser, I. (1990) Cell 60, 225–234
9. Novick, D., Cohen, B., and Rubinstein, M. (1994) Cell 77, 391–400
10. Colamonici, O. R., Yan, H., Domanski, P., Handa, R., Smalley, D., Mullersman, J., Witte, M., Krishnan, K., and Królewski, J. J. (1994) Mol. Cell. Biol. 14, 8133–8142
11. Constantinescu, S. N., Cróze, E., Wang, C., Murti, A., Basu, L., Mullersman, J. E., and Pfeffer, L. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9602–9606
12. Uezu, G., Lutfiafla, G., and Mogensen, K. E. (1995) J. Interferon Cytokine Res. 15, 3–26
13. Platanias, L. C., Pfeffer, L. M., Barton, K. P., Vardinman, J. W., Golomb, H. M., and Colamonici, O. R. (1992) Br. J. Haematol. 82, 543–546
14. Platanias, L. C., and Colamonici, O. R. (1993) J. Biol. Chem. 268, 24053–24057
15. Platanias, L. C., Ubeda, S., and Colamonici, O. R. (1994) J. Biol. Chem. 269, 17761–17764
16. Colamonici, O. R., Uyttendaele, H., Domanski, P., Yan, H., and Królewski, J. J. (1994) J. Biol. Chem. 269, 3518–3522
17. Platanias, L. C., and Sweet, M. E. (1994) J. Biol. Chem. 269, 3143–3146
18. Uddin, S., Yenush, L., Sun, X-J., Sweet, M. E., White, M. F., and Platanias, L. C. (1995) J. Biol. Chem. 270, 15938–15941
19. Darnell, J. E., Kerr, I. M., and Stark, G. R. (1994) Science 264, 1415–1421
20. Fu, X-Y. (1992) Cell 70, 323–335
21. Schindler, C., Shuai, K., Preziosi, V. R., and Darnell, J. E., Jr. (1992) Science 257, 809–813
22. Gutch, M. J., Daly, C., and Reich, N. C. (1992) Proc. Natl. Acad. Sci. U. S. A. 8, 11411–11415
23. Heim, M. H., Kerr, I. M., Stark, G. R., and Darnell, J. E., Jr. (1995) Science 267, 1347–1353
24. Leung, S., Qureshi, S. A., Kerr, I. M., Darnell, J. E., Jr., and Stark, G. R. (1995) Mol. Cell. Biol. 15, 1312–1317

2 S. Uddin, A. Chamdin, and L. C. Platanias, unpublished data.
Interaction of the Transcriptional Activator Stat-2 with the Type I Interferon Receptor
Shahab Uddin, Aghiad Chamdin and Leonidas C. Platanias

J. Biol. Chem. 1995, 270:24627-24630.
doi: 10.1074/jbc.270.42.24627

Access the most updated version of this article at http://www.jbc.org/content/270/42/24627

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 24 references, 17 of which can be accessed free at http://www.jbc.org/content/270/42/24627.full.html#ref-list-1