A previous study using random mutagenesis identified an activating mutation in the common β subunit (hβc) of the human granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5 receptors in which an isoleucine residue (Ile374) in the extracellular region of hβc is replaced by asparagine (Jenkins, B. J., D'Andrea, R., and Gonda, T. J. (1995) EMBO J 14, 4275–4287). To investigate the mechanism by which this mutation (I374N) acts, we employed site-directed mutagenesis to explore predictions based on a structural model of hβc. We focused on possible interactions between Ile374 and other hydrophobic residues in its vicinity and found that replacement of two such residues, Leu356 and Trp358, with asparagine resulted in constitutive activation of hβc. Hydrophilic substitutions at both of these positions and at position 374 resulted in the greatest degree of activation, as measured by the growth rate of factor-independent cells, while hydrophobic substitutions had lesser or no effects. Moreover, these “weak” substitutions appeared to synergize, since disruptive substitutions had lesser or no effects. Furthermore, the high-affinity receptors for human GM-CSF (hGMR), IL-3 (hIL-3R), and IL-5 (hIL-5R) consist of specific ligand-binding α subunits (hGMα, hIL-3Rα, and hIL-5Rα) associated with a common signal-transducing β subunit (hβc). The α subunits bind their cognate factors with low affinity, whereas hβc does not bind any cytokine by itself, but is required to confer high-affinity binding when co-expressed with the α subunits (1–5). The hGMRα, hIL-3Rα, hIL-5Rα, and hβc subunits belong to the cytokine receptor family which includes the receptors for many hemopoietic growth factors and other cytokines such as growth hormone (GH), erythropoietin, thrombopoietin (or c-Mpl ligand), IL-2 and IL-6 (reviewed in Ref. 6). Members of this family are characterized by an extracellular cytokine receptor module of about 200 amino acids containing several conserved motifs, including the hallmark WSXWS (Trp-Ser-Xaa-Trp-Ser) motif (7).

The mechanism by which cytokine binding induces receptors to form active signal-transducing complexes has not been fully elucidated, but there is increasing evidence to suggest that ligand-induced dimerization of receptor subunits is an essential early step in receptor activation. To date, the best-characterized active cytokine receptor complex is that of the human GH receptor (hGHR), the tertiary structure of which has been elucidated by x-ray crystallography (8). The active hGHR complex exists as a homodimer in which both hGHR subunits interact with each other and a single GH molecule. Indirect evidence that homodimerization of the receptors for erythropoietin receptor and thrombopoietin (c-Mpl) is similarly essential for signaling has arisen from the isolation of constitutively activated mutants which most likely mimic ligand-induced homodimerization of these receptors (9–11).

The receptors for a number of other cytokines are more complex and are comprised of two or more distinct subunits. For example, the active IL-6 receptor complex consists of two IL-6 molecules associated with two IL-6-specific α subunits and a dimer of the common signaling subunit, gp130 (12–14). The complexity of active cytokine receptor structures is further epitomized by the IL-2 receptor, in which IL-2 induces association of a ligand-binding α subunit and two distinct signaling subunits, β and γ (15).

With regard to the hGMR, hIL-3R, and hIL-5R, the precise composition or stoichiometry of the active receptor complexes has yet to be determined, although it is becoming clear that hGMR (16) and hIL-3R (17) undergo ligand-induced αβ heterodimerization. However, several chimeric receptors that contain the cytoplasmic domain of the β subunit and are predicted to undergo ligand-dependent dimerization have been shown to induce cellular proliferation (17–20). Moreover, we have previously identified an activating mutation in the transmembrane domain of hβc (V449E) which, by analogy with a similar mutation in the neu oncogene (21–23), may act by inducing homodimerization of hβc (24). Taken together, these data imply that dimerization of the intracellular portion of the β subunit is essential early step in receptor activation.
sufficient to initiate cellular proliferation; however, it has not yet been demonstrated that normal GMR, IL-3R, or IL-5R complexes do in fact contain β subunit dimers.

Our previous work has identified two other activating mutations in hβc. One mutant, I374N (24), substitutes Asn for Ile at position 374 in the membrane-proximal extracellular domain of hβc, while F132 contains a small duplication in this same domain (25). Unlike V449E, neither of these extracellular mutants could confer factor independence on the BAF-B03 cell line, implying that they activate hβc by a different mechanism (24). These findings raise two questions: (i) how do the I374N (and other extracellular) mutation(s) alter hβc structure and lead to constitutive receptor activation; and (ii) what features of the activated receptor complexes formed by the transmembrane and extracellular mutants are responsible for their different cell-type specificities. In the present report, we address the first of these questions. Initially, we examined the specific structural requirements for receptor activation at residue 374 by introducing a range of amino acid substitutions at this position and testing their ability to confer factor independence on a factor-dependent hematopoietic cell line. We then employed molecular modeling of hβc to predict which residues might interact with Ile374. We show that amino acid substitutions at some of these residues lead to constitutive activation of hβc, thus implicating them in receptor activation and interaction with Ile374. These findings lead us to propose a model in which interactions between residues in the membrane-proximal region of hβc are involved in both normal and constitutive receptor activation.

**EXPERIMENTAL PROCEDURES**

**Molecular Modeling of hβc—**A model of the fourth domain of hβc was developed based on the crystal structure coordinates of the human growth hormone-binding protein (8). The sequences of hβc and domain 2 of the GH-binding protein II were aligned manually and an Indigo computer (Silicon Graphics) was used to run the molecular modeling programs Insight II, Homology, and Discover (Molecular Simulations Inc., San Diego, CA). Coordinates for regions of hβc thought to be conserved structurally, corresponding to the proposed β-strands, were assigned from the homologous backbone coordinates of GH-binding protein II and some side chain coordinates. Additional loops were assigned from coordinates from a library of protein structures. An inspection of the model revealed a well packed hydrophobic core with only moderate steric clashes between the hydrophobic side chains of adjacent strands. Manual and automated methods were used to select appropriate conformations for the hydrophobic side chains of residues proposed to be buried in the core of the hβc molecule. The model was evaluated for stereochemical parameters using Procheck (26) and is shown in Figure 1A. The sequences corresponding to the predicted B, C, and F β-strands of hβc are shown in Figure 1B.

**Site-directed Mutagenesis and Construction of Expression Plasmids—**The hβc cDNA used for mutagenesis was that described by Barry et al. (27); amino acids are numbered from the initiating methionine. Site-directed mutagenesis was carried out on single-stranded DNA with mutagenic oligonucleotides using the pALTER-1 system (Promega) in accordance with the manufacturer’s instructions. All mutations were confirmed by DNA sequencing, following which mutant hβc cDNAs were subcloned between the BamHI and HindIII restriction sites of the pRUFNeo retroviral expression vector (28).

**Cell Lines and Cell Culture—**V2 (29) and BOSC 23 (30) retroviral packaging cell lines were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. V2 cells producing either wild-type hGMRa retrovirus (24) or hIL-3Ra retrovirus (24) were received from the laboratory of Dr. Barry and Clive Barry, respectively. A retrovirus (24) containing a vector containing the human IL-3 receptor α chain was kindly provided by Dr. Barry. HMGaR (Silenus) and hGMR (24) were kindly provided by Dr. Barry. Anti-hβc monoclonal antibodies 6H6 and 8G6 (35) and 6H6 (36), respectively.

**Results**

Predicted Structure and Molecular Modeling of hβc—**Determination of the three-dimensional structure of the hGH (8) together with sequence alignments and structural prediction (7) has lead to the definition of the cytokine receptor module as a fundamental structural unit of the cytokine receptor family. The structure within this module is conserved and consists of two folding subdomains, each containing seven anti-parallel β-strands (identified by the letters A–G). These are folded into a “sandwich” comprised of two sheets, with the first containing β-strands A, B, and E, and the second containing strands C, D, F, and G (7, 8, 39). We have used sequence alignment of the extracellular region of hβc with hGHR and other members of the cytokine receptor family, and comparison with the structure of the hGH-hGHR complex, to derive a molecular model of the membrane-proximal cytokine receptor module of hβc (8).

**Biological Activity of Ile374 hβc Mutants—**Expression in FDC-P1 Cells—**We have previously reported that substitution of asparagine for isoleucine at residue 374 (I374N) in domain 4 of hβc (24) resulted in constitutive activation as shown by its ability to confer factor-independent growth on the factor-dependent hematopoietic cell line, FDC-P1. The model illustrated in Fig. 1A suggests that the Ile374 residue lies on the C strand. 

2 C. J. Bagley and A. F. Lopez, manuscript in preparation.
and is buried within a hydrophobic region in domain 4 of hβc. Furthermore, sequence alignment of the extracellular region of hβc with hGHR and other members of the cytokine receptor superfamily suggest that the hydrophobic nature of the Ile374 residue in hβc is highly conserved at the corresponding positions among members of this receptor family (Fig. 1B). Based upon these observations, it is likely that the hydrophilic substitution in I374N would severely distort the conformation of this region.

To further examine the structural requirements for receptor activation, site-directed mutagenesis was used to replace Ile374 with a series of different amino acids (alanine, phenylalanine, glutamine, and aspartic acid) which were predicted to induce increasing degrees of structural disruption. The Ile374 hβc mutant cDNAs were inserted into the pRUFNeo retroviral vector and introduced into FDC-P1 cells, which were then selected for G418 resistance. Flow cytometric analysis of G418-selected clones (isolated as colonies in soft agar) that expressed comparable levels of each mutant. Comparison of these factor-independent FDC-P1 clones indicated that clones expressing I374F also proliferated at a significantly lower rate than those expressing the I374D, I374Q, and I374N mutants (data not shown).

Constitutive Activation of Wild-type hβc upon Substitutions at Leu356 or Trp358 Residues—We used a molecular model of domain 4 of hβc (Fig. 1A) to identify the residues within this domain of hβc most likely to interact with Ile374. Using this approach, four residues, Leu356, Trp358, Val358, and Val354 were predicted to interact with Ile374. The two Val residues are located on the F strand in the same β-sheet as Ile374 (on strand C), whereas the Trp and Leu residues are located on the B strand in the opposing, second β-sheet (Fig. 1, A and B). Interestingly, sequence alignment of the extracellular regions of hβc and other members of the cytokine receptor family indicate that the Trp358 residue is highly conserved among other cytokine receptors. These alignments also indicate that the hydrophobicity of residues corresponding to the Leu356 position is conserved in other cytokine receptors, and that the Val354 and Val358 residues are part of a conserved motif, “RVVR” in hβc, typified by an Arg-Val-Arg sequence (25, 40) (Fig. 1B).

We reasoned that if, as predicted, the residues at positions 356, 358, 412, and 414 normally interacted with Ile374, then substitutions at some or all of these positions might also disrupt these interactions and result in receptor activation. Since strong activation occurred by replacing the hydrophobic Ile374 residue with asparagine, we first tested the effect of this substitution at each of the potentially interacting positions to generate the mutants L356N, W358N, V412N, and V414N.
Retroviruses encoding these mutants, as well as wild-type hβc, were used to infect FDC-P1 cells which were then selected for G418 resistance in liquid culture. Initial flow cytometric analyses of the resultant cell populations indicated that cell-surface expression of some hβc mutants was only detectable by high-sensitivity immunofluorescence (data not shown); as a result, subsequent flow cytometric analyses were performed on cells stained with this method (see “Experimental Procedures”). Fig. 4A shows that all of these mutants (as well as I374N, I374F, and wild-type hβc) were expressed by the G418-resistant cell populations. After several weeks selection for factor independence in liquid culture, only cultures infected with the Leu356, Trp358, or Ile374 hβc mutants produced colonies that grew without mGM-CSF. Although the frequency of factor independence for cells infected with each activated mutant was low, this again is likely to be due to the fact that only a subset of G418-selected cells expressed the hβc mutants on the cell surface (Fig. 4A). Factor independence was not a result of low-level autocrine growth factor production as conditioned medium from the factor-independent cell pools did not support the growth of uninfected FDC-P1 cells (data not shown). Additionally, the presence of the appropriate full-length hβc mutant cDNAs in the infected FDC-P1 cells was confirmed by recovery of the entire hβc fragment by polymerase chain reaction from genomic DNA, followed by restriction enzyme digestions diagnostic of each mutant (data not shown).

The rate of factor-independent proliferation of cells expressing the L356N and W358N mutants was considerably lower than that seen with I374N, as shown by proliferation assays (Fig. 5A). Furthermore, the proliferation rates of factor-independent cell populations infected with the L356A and W358F mutants were severalfold lower even than those of cells infected with the L356N and W358N mutants, although surface expression was slightly higher for the former two mutants (Fig. 5B). Interestingly, the factor-independent colonies (as in Fig. 4B) that arose from FDC-P1 cells infected with the L356A and W358F mutants were significantly smaller than those infected with the other activated mutants (data not shown). Together, these results suggest that the Leu356 and Trp358 mutants induced constitutive activation less efficiently than the Ile374 mutants and that the asparagine substitutions at these positions lead to higher activity than the more hydrophobic (alanine and phenylalanine) substitutions.
Substitutions at Positions 356 or 358 Can Synergistically Enhance Activation of the I374F Mutant—An extension of the notion that disruption of interactions between Ile 374 and Leu356/Trp358 leads to constitutive activation is that weakly activating mutations at both of the interacting positions might synergize in enhancing receptor activation. We therefore constructed four double mutants by combining I374F with L356A, W358F, and also with glycine substitutions for each of the two valine residues at positions 412 and 414. The latter double mutants (I374F/V412G and I374F/V414G) were constructed to provide negative controls for synergy. Glycine was chosen as a smaller, non-polar residue to replace valine, in a similar vein to replacing leucine with alanine and tryptophan with phenylalanine in L356A and W358F, respectively; it was unlikely that these substitutions would be activating as even the disruptive asparagine substitutions for Val412 or Val414 did not result in activation (Fig. 4B). All four double mutants conferred factor independence upon FDC-P1 cells; however, the proliferation rates of factor-independent cells infected with the I374F/L356A and I374F/W358F mutants were consistently severalfold higher than those infected with the I374F/V412G, I374F/V414G, or I374F mutants (Fig. 6A). Indeed, the proliferation rates seen with the I374F/L356A and I374F/W358F mutants were similar to that of the strongly-activated I374N mutant. In contrast, the proliferation rates of cells expressing the I374F/V412G and I374F/V414G mutants barely differed from that of cells expressing the “parental” I374F single mutant. These differences in growth rates could not be attributed to corresponding differences in the level of cell-surface expression of the various mutants (Fig. 6B). Thus these data, and the data of Figs. 4 and 5, are consistent with the notion that activation of h\(\beta\)c by the Ile374, Leu356, and Trp358 mutants is due to disruption of the interactions between Ile374 and the latter two residues.

Biological Activity of L356N and W358N Mutants in BAF-B03 Cells—We have previously shown that the I374N mutant, while constitutively activated in FDC-P1 cells, is unable to confer factor independence on mouse IL-3-dependent BAF-B03 cells (24). In contrast, another previously identified h\(\beta\)c mutant, V449E (24), is constitutively activated in both cell types. We reasoned that if the L356N and W358N mutants are activated in FDC-P1 cells by the same mechanism as I374N, then these mutants would also be unable to confer factor independence on BAF-B03 cells. Retroviruses encoding the wild-type and mutant forms of h\(\beta\)c were therefore used to infect BAF-B03 cells. As shown in Fig. 7A, each mutant was expressed on the cell surface of infected cells. However, proliferation assays

**Fig. 4.** Expression of, and factor-independent colony formation by, FDC-P1 cells infected with h\(\beta\)c mutants. A, flow cytometric analysis of mutant h\(\beta\)c expression on G418-selected FDC-P1 cells. Cells were stained by high-sensitivity immunofluorescence; dashed lines represent cells stained with an irrelevant control antibody and solid lines indicate staining with the anti-h\(\beta\)c antibody 1C1. Axes are as in Fig. 2A. For comparison, analyses of uninfected cells and cells infected with wild-type h\(\beta\)c are also shown. B, frequency of factor independence of FDC-P1 cells infected with h\(\beta\)c mutants. Cells were washed and plated in agar-containing medium with or without mouse GM-CSF immediately after co-cultivation with transiently transfected BOSC 23 cells. Data are presented as the average number of colonies present on duplicate agar plates seeded with 500 or 5000 cells. Percentage of factor independence is calculated as the percentage of infected, i.e. G418-resistant (as determined from plates seeded with 500 cells), colonies that grew on plates, seeded with 5000 cells, in the absence of mGM-CSF.

**Fig. 5.** Proliferation of factor-independent FDC-P1 cells infected with activated h\(\beta\)c mutants. A, proliferation assay of FDC-P1 cells, infected with the indicated h\(\beta\)c mutants, which had been selected prior to assay for growth in the absence of factor. Also shown are uninfected cells that were washed and assayed in medium without mouse GM-CSF. The inset shows an enlargement of the proliferation profiles of uninfected cells and cells expressing the L356A and W358F mutants. Procedures and axes are as in Fig. 3A. B, flow cytometric analysis of activated h\(\beta\)c mutant expression on the factor-independent FDC-P1 cells depicted in A. Procedures, nomenclature, and axes are as in Fig. 4A.
showed that none of these mutants were able to confer factor independence on BAF-B03 cells in liquid culture (Fig. 7B). Although the I374N mutant is not constitutively activated in BAF-B03 cells, it is still capable of forming a high-affinity receptor and delivering a proliferative signal, in the presence of human GM-CSF, when co-expressed with the hGMRα subunit (24). We therefore examined the ability of the L356N and W358N mutants to behave as wild-type β subunits by super-infecting BAF-B03 cells expressing these mutants with a retrovirus encoding the hGMRα subunit. Flow cytometric analysis indicated that the hGMRα subunit was efficiently co-expressed with the wild-type and mutant β subunits on the surface of infected cells (Fig. 7A). However, only cells expressing the wild-type, and as expected I374N mutant β subunits were able to proliferate in 0.1 ng/ml hGM-CSF, as shown by proliferation assays (Fig. 7B), and prolonged monitoring of liquid cultures in the presence of either 0.1 or 1 ng/ml hGM-CSF.

To determine whether this lack of proliferation resulted from a loss of high-affinity binding associated with the L356N and W358N mutants, saturation binding studies were performed on cells with 125I-labeled GM-CSF expressing hGMRα alone, and hGMRα with either the I374N or W358N mutant. As shown in Fig. 7C, cells co-expressing hGMRα and I374N exhibited both low-affinity ($K_d = 2 \text{ nM}$) and high-affinity ($K_d = 78 \text{ pM}$) binding sites. These binding affinities are consistent with those previously determined for cells co-expressing wild-type hGMRα and β subunits (2, 35). In contrast, only low-affinity binding sites were detected on cells co-expressing hGMRα and W358N ($K_d = 2.1 \text{ nM}$), or as expected, cells expressing hGMRα alone ($K_d = 1.6 \text{ nM}$). We have also examined the ability of BAF-B03 cells co-expressing the L356N or W358N mutants with the hIL-3Rα subunit to proliferate in response to hIL-3. Although flow cytometric analysis indicated that the hIL-3Rα subunit was efficiently cell-surface expressed with wild-type and mutant β subunits, only cells expressing wild-type or I374N mutant β subunits proliferated in response to 1 ng/ml hIL-3 (data not shown).

3 B. J. Jenkins, Q. Sun, A. F. Lopez, and T. J. Gonda, unpublished observations.

4 B. J. Jenkins, Q. Sun, J. Woodcock, A. F. Lopez, and T. J. Gonda, unpublished results.
We have previously reported that hβc can be rendered constitutively active by a point mutation in the extracellular region which replaces a conserved isoleucine residue at position 374 with asparagine (I374N) (24). As one way of exploring the mechanism by which this mutation (and possibly other mutations in domain 4), acts, we have utilized a molecular model of part of the extracellular portion of hβc to design further mutants. In particular, we have focused on possible interactions between IleE774 and other, neighboring residues in the predicted hβc structure.

One of the key observations in this work was that replacement by asparagine of Trp356 or Leu358, which are predicted to participate in van der Waals interactions with IleE774, also resulted in activation. Potentially less disruptive mutants, in which these residues were replaced with phenylalanine or alanine (in W358F and L356A, respectively), were very weakly activating by themselves. However, we found that these relatively mild changes greatly enhanced factor-independent proliferation when combined with a relatively weak mutation (I374P) at position 374. The results support the prediction that IleE774 interacts with LeuE358 and TrpE356, and lead us to suggest that (i) these interactions are normally involved in maintaining the conformation of domain 4, and (ii) that disruption of these interactions leads to a conformational change which results in receptor activation. While the implications of such a model are discussed further below, we will consider several other observations that support this interpretation of our results.

First, substitutions at position 374 other than the original asparagine resulted in activation, with those expected to be most disruptive, i.e. other hydrophilic residues, resulting in maximal activation as judged by the growth rates of factor-independent cells. Second, a similar pattern holds for substitutions at positions 356 and 358 in that the asparagine substitutions induced far greater factor-independent growth than the alanine (L356A) or phenylalanine (W358F) substitutions. Third, we note that the interacting residues IleE774 and TrpE356/LeuE358 are predicted to lie on β-strands C and B, respectively, and so we could generalize that other interactions between these two strands may also be important in maintaining the normal structure of domain 4. Indeed, data from random mutagenesis have shown that TyrE376 in strand C is also a target for activating mutations. Finally, the fact that like I374N, neither the W358N nor the L356N mutant could confer factor independence on BAF-B03 cells is consistent with a common mode of action.

One rather unexpected result of these studies was that neither the W358N nor the L356N mutant could form a high-affinity receptor on, or elicit hGM-CSF-dependent proliferation of, BAF-B03 cells expressing the hGMαR subunit; this is in contrast to I374N which exhibits wild-type function under these conditions. Two possible explanations are that the W358N and L356N mutations prevent interaction with either GM-CSF itself or with the hGMαR subunit. The first of these is consistent with the finding of Woodcock et al. (35) that residues adjacent to β-strand B, in the B-C loop, were necessary for high-affinity hGM-CSF binding. Similarly, the inability of the W358N and L356N mutants to allow proliferation in response to hIL-3 in the presence of the hIL-3Rα subunit is consistent with the recent finding that other mutations in the B-C loop interfere with IL-3 binding. However, it is equally possible that these mutations prevent functional interaction with receptor α subunits; this would be consistent with the prediction that contacts between the α and β subunits involve the adjacent A-B loop (8).

 Ideally, we would like to integrate our observations into a model that could explain how the activating mutations achieve their effect and how this mechanism relates to normal receptor function. Unfortunately, it is not yet known precisely how the GMR is triggered by ligand binding, nor is the stoichiometry of the active receptor complex known (other than that it contains at least one α subunit and one β subunit). It is highly likely, however, that the active normal GMR complex contains more than one signaling subunit, as do the active forms of all other known cytokine receptors; these could be either two β subunits or, as we have suggested elsewhere (24) a β subunit plus a putative heterologous signaling subunit (“γ”), and thus triggers receptor signaling. Note that for the sake of clarity, the ligand itself is not depicted. C and D, activating mutations in β-strands B or C (depicted by asterisks) disrupt interactions between the two β-sheets and result in sheet 2 assuming an activated conformation, which in turn allows interaction with the second signaling subunit (as in part B).

As mentioned previously, structural modeling of the α-β-ligand complex indicates that domain 4 of hβc can be viewed as two β-sheets, one comprised of strands A, B, and E (β-sheet 1), and the second comprised of strands D, C, F, and G (β-sheet 2) (8, 39). This model, which is supported by extensive studies on the interactions between GM-CSF (and IL-3) and both the α and β subunits (e.g. Ref. 41), predicts that β-strand E and the A-B loop in β-sheet 1 contact the α subunit (see Figs. 1 and 8B). Thus, we would predict that interactions with a second signaling subunit would take place via the opposite “side” of domain 4, i.e. β-sheet 2 (see Fig. 8B). In view of this and the results presented in this report, we propose the following model, illustrated in Fig. 8, for the role of domain 4 in the activation of hβc. In both inactive and active forms of the wild-type receptor, β-sheets 1 and 2, and specifically strands B and C interact via contacts including those between IleE774 and TrpE356/LeuE358 (Fig.

**FIG. 8. Model for the involvement of interactions between β-strands B and C of domain 4 in receptor activation.** A, in the inactive, i.e. uncomplexed form, interactions (double arrow) between the two β-sheets, comprising β-strands A, B, and E (β-sheet 1) and β-strands C, D, F, and G (β-sheet 2), respectively, stabilize the inactive conformation of domain 4. B, interaction with α subunit plus ligand induces a conformational change in β-sheet 1 that is transmitted via the B-C interaction to generate a conformational change in β-sheet 2; the altered conformations are represented by increased curvature (compared to A). The altered conformation of β-sheet 2 results in association with a second signaling subunit (either another β subunit, β’, or a possible heterologous signaling subunit (“γ”), and thus triggers receptor signaling. Note that for the sake of clarity, the ligand itself is not depicted. C and D, activating mutations in β-strands B or C (depicted by asterisks) disrupt interactions between the two β-sheets and result in sheet 2 assuming an activated conformation, which in turn allows interaction with the second signaling subunit (as in part B).

---

5 B. J. Jenkins and T. J. Gonda, unpublished observations.
6 J. Woodcock and A. F. Lopez, unpublished observations.
8, A and B). Association with the α subunit plus ligand induces a conformational change in β-sheet 1 or a rearrangement of the interface between the two sheets, and this is transmitted via the B-C interaction to the second β-sheet (Fig. 8B). The ensuing conformational change (to which contacts between ligand and hβc could also contribute) then promotes interaction of residues in this β-sheet with the second signaling subunit, leading to dimerization and triggering of intracellular signaling pathways. In the case of the activating mutations affecting Ile374, Trp358, and Leu256, disruption of the B-C interaction would lead to the second β-sheet assuming an activated conformation similar to that seen after a subunit/ligand binding in the normal receptor (Figs. 8, C and D). Validation or rejection of this model will ultimately require definition of the subunit composition of both wild-type and mutant GMR/IL-3R/IL-5R complexes, and identification of all surfaces participating in intersubunit interactions.

Finally, we note that the three interacting residues studied in this report, Ile374, Leu256, and Trp358, are highly conserved within the cytokine receptor family (e.g. see Fig. 1B). Thus, the homologous residues in other cytokine receptors may be targets for activating mutations and, furthermore, the model proposed here may also be applicable to other receptors.

Acknowledgments—We thank Dr. Richard D’Andrea for valuable discussions and access to unpublished results, Dr. Heddy Zola (Women’s and Children’s Hospital, Adelaide) for advice and assistance with high-sensitivity immunostaining, and Alan Bishop and Judy Haywood for assistance with flow cytometry.

REFERENCES

1. Gearing, D. P., King, J. A., Gough, N. M., and Nicola, N. A. (1989) EMBO J. 8, 3667–3676
2. Hayashida, K., Kitamura, T., Gorman, D. M., Ariai, K., Yokota, T., and Miyajima, A. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 9655–9659
3. Kitamura, T., Sato, N., Ariai, K., and Miyajima, A. (1991) Cell 66, 1165–1174
4. Tavernier, J., Devos, R., Cornelis, S., Tuybens, T., der Hayden, J. V., Fiers, W., and Plaetinck, G. (1991) Cell 66, 1175–1184
5. Sakamaki, K., Miyajima, I., Kitamura, T., and Miyajima, A. (1992) EMBO J. 11, 3541–3549
6. Mui, A. L., and Miyajima, A. (1994) Prog. Growth Factor Res. 5, 15–35
7. Bazan, J. F. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 6934–6938
8. De Vos, A. M., Ullrich, A., and Russkikh, A. A. (1992) Science 255, 306–312
9. Alexander, W. S., Metcalf, D., and Dunn, A. R. (1995) EMBO J. 14, 5569–5578
10. Yoshimura, A., Longmore, G., and Lodish, H. F. (1990) Nature 348, 647–649
11. Watowich, S. S., Yoshimura, A., Longmore, G. D., Hilton, D. J., Yoshimura, Y., and Lodish, H. F. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 2140–2144
12. Ward, L. D., Howlett, G. J., Discole, G., Yasukawa, K., Hammacher, A., Moritz, R. L., and Simpson, R. J. (1994) J. Biol. Chem. 269, 23286–23299
13. Ponzessa, G., Graziani, R., De Serio, A., Savino, R., Ciapponi, L., Lahm, A., Salvai, A. L., Tongiatt, C., and Gilbert, G. (1995) EMBO J. 14, 1942–1951
14. Murakami, M., Hibi, M., Nakagawa, N., Nakagawa, T., Yasukawa, K., Yamanishi, K., Taka, T., and Kishimoto, T. (1993) Science 260, 1808–1810
15. Taniguchi, T., and Minami, Y. (1993) Cell 73, 5–8
16. Eder, M., Erns, T. J., Vanier, A., Juhinsky, P. T., Ihnorn, R., Hoelzer, D., and Griffin, J. D. (1994) J. Biol. Chem. 269, 30173–30180
17. Stomski, F. C., Sun, Q., Bagley, C. J., Woolcock, J. M., Goodall, G. J., Andrews, T. R., Berndt, M. C., and Lopez, A. F. (1994) Mol. Cell Biol. 14, 7404–7413
18. Takaki, S., Kanazawa, H., Shibai, M., and Takatsu, K. (1994) Mol. Cell Biol. 14, 7404–7413
19. Sakamaki, K., Wang, H. M., Miyajima, I., Kitamura, T., Todokoro, K., Harada, N., and Miyajima, A. (1993) J. Biol. Chem. 268, 15833–15839
20. Muto, A., Watanabe, S., Miyajima, A., Yokota, T., and Ariai, K. (1995) Biochem. Biophys. Res. Commun. 208, 368–375
21. Bergmann, C. I., Hung, M.-C., and Weinberg, R. A. (1986) Nature 319, 226–230
22. Weiner, D. B., Liu, J., Cohen, J. A., Williams, W. V., and Greene, M. I. (1989) Nature 339, 200–211
23. Sternberg, M. E., and Gullick, W. J. (1989) Nature 339, 587
24. Jenkins, B. J., D’Andrea, R., and Gonda, T. J. (1995) EMBO J. 14, 4276–4287
25. D’Andrea, R., Rayner, J., Moretti, P., Lopez, A., Goodall, G. J., Gonda, T. J., and Vadas, M. (1994) Blood 83, 2802–2808
26. Laskevskii, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) J. Appl. Cryst. 26, 283–291
27. Barry, S. C., Bagley, C. J., Phillips, J., Dottore, M., Cambaresi, B., Moretti, P., D’Andrea, R., Goodall, G. J., Shannon, M. F., Vadas, M. A., and Lopez, A. F. (1994) J. Biol. Chem. 269, 8488–8492
28. Rayner, J., and Gonda, T. J. (1994) Mol. Cell. Biol. 14, 880–887
29. Mann, R., Mulligan, R. C., and Baltimore, D. (1983) Cell 33, 153–159
30. Pear, W. S., Nolan, G. P., Scott, M. L., and Baltimore, D. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 8390–8394
31. Dexter, T. M., Garland, J., Scott, D., Seolnick, R., and Metcalfe, D. (1980) J. Exp. Med. 152, 1036–1047
32. Hatakeyama, M., Mori, H., Nai, T., and Taniguchi, T. (1989) Cell 59, 837–845
33. Johnsen, G. R. (1980) J. Cell Physiol. 103, 371–383
34. Zola, H., Neoh, S. H., Mantziaris, B. X., Webster, J., and Loughman, N. M. (1990) J. Immunol. Methods 133, 247–255
35. Woodcock, J. M., Zacharakis, B., Plaetinck, G., Bagley, C. J., Qiu, S., Hersus, T. R., Tavernier, J., and Lopez, A. F. (1994) EMBO J. 13, 5176–5185
36. Sun, Q., Woodcock, J. M., Rapoport, A., Stomski, F. C., Koripelainen, E. I., Bagley, C. J., Goodall, G. J., Smith, W. B., Gamble, J. R., Vadas, M. A., and Lopez, A. F. (1996) Blood 87, 83–92
37. Contreras, M. A., Bale, W. F., and Spar, I. L. (1983) Methods Enzymol. 92, 277–292
38. Mumper, P. J., and Roebard, D. (1980) Anal. Biochem. 107, 220–239
39. Goodall, G. J., Bagley, C. J., Vadas, M. A., and Lopez, A. F. (1995) Growth Factors 8, 87–97
40. Patthy, L. (1990) Cell 61, 13
41. Bagley, C. J., Woodcock, J. M., Hercus, T., Shannon, M. F., and Lopez, A. F. (1995) J. Leukocyte Biol. 57, 739–746
42. Kraulis, P. (1991) J. Appl. Cryst. 24, 946–950
43. Merritt, E. A., and Murphy, M. E. (1994) Acta. Cryst. Sect. D 50, 869–873