Structural Basis for Binding Multiple Ligands by the Common Cytokine Receptor γ-Chain*

The common γ-chain (γc) that functions both in ligand binding and signal transduction is a shared subunit of the multichain receptors for interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21. The structural basis by which the ectodomain of γc contributes to binding six distinct cytokines is only partially defined. In the present study, epitope mapping of antagonistic anti-γc monoclonal antibodies led to the identification of Asn-128 of mouse γc that represents another potential contact residue that is required for binding IL-2, IL-7, and IL-15 but not IL-4. In addition, Tyr-103, Cys-161, Cys-210, and Cys-211, previously identified to contribute to binding IL-2 and IL-7, were also found to be involved in binding IL-4 and IL-15. Collectively, these data favor a model in which γc utilizes a common mechanism for its interactions with multiple cytokines, and the binding sites are largely overlapping but not identical. Asn-128 and Tyr-103 likely act as contact residues whereas Cys-161, Cys-210, and Gly-211 may stabilize the structure of the proposed ligand-interacting surface formed by the two extracytoplasmic domains.

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Ferenc Olosz and Thomas R. Malek‡
From the Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, Florida 33101

‡ To whom correspondence should be addressed: Dept. of Microbiology and Immunology (R138), University of Miami School of Medicine, P.O. Box 016960, Miami, FL 33101. Tel.: 305-243-5627; Fax: 305-243-4623; E-mail: tmalek@med.miami.edu.

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The abbreviations used are: γc, common γ-chain; m, mouse; R, receptor; FACS, fluorescence-activated cell sorter; h, human; IL, interleukin; RT, reverse transcription; WT, wild-type; X-SCID, X-linked severe combined immunodeficiency disease; mAb, monoclonal antibody.

Lymphocyte development, self-reactivity, T cell homeostasis, and peripheral immune responses are importantly regulated by cytokines that utilize the common γ-chain (γc) as a receptor subunit (1, 2). γc is a type I transmembrane glycoprotein that serves as a subunit for the receptors of interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21. The IL-2 receptor (R) and IL-15Rα heterotrimers comprised of unique α-chains and shared IL-2Rβγ and γc subunits whereas the IL-4R, IL-7R, IL-9R, and IL-21R are heterodimers comprised of unique α-subunits and γc (3–9). For each of these cytokine receptors, γc directly contributes to ligand binding through its extracellular domain and to signal transduction through the association of Jak-3 to its cytoplasmic tail (10, 11).

Mutations in γc abolish the function in each of these cytokine receptors and cause X-linked severe combined immunodeficiency disease (X-SCID) (12, 13). In humans this disease is characterized by failed development of T and natural killer cells whereas B cell development is largely normal, but their function is impaired. In γc-deficient mice, severe impairment in T, natural killer, and B cell development occurs that is mainly due to lack of IL-7R and IL-15R signaling (14). A number of mutations in γc from X-SCID patients have been identified (15, 16). Many of these are nonsense mutations that lead to a premature stoppage in translation of γc whereas several others are missense mutations. For the most part, however, it is not possible to deduce whether these latter mutations might selectively affect the ligand-binding function of γc or simply distort its tertiary structure.

The structural basis by which γc functions in binding six distinct cytokines is still not defined. Recent structure-function analysis of the mouse γc (mγc) ectodomain by site-directed mutagenesis (17), guided by predictions of theoretical models of the γc structure (18–20), identified four amino acids of γc that are necessary for binding IL-2 and IL-7 (17). One of these residues, Tyr-103, may be directly involved in IL-2 and IL-7 binding. The other three residues, Cys-161, Cys-210, and Gly-211 may indirectly function in ligand binding by maintaining the conformation of two proposed loops of γc. Somewhat surprisingly, none of the initial 25 mutations introduced into mγc substantially affected the binding of two well characterized antagonistic anti-mγc mAbs (4G3 and 3E12) (21) suggesting that there remained other important ligand contact regions in γc.

In the present report we have defined the epitopes on mγc for the 4G3 and 3E12 mAbs. Subsequent site-directed mutagenesis of amino acids surrounding the putative 4G3 binding site led to the identification of a novel residue that is more critical for the binding of IL-7 than IL-2 or IL-15 and is not important for binding IL-4. In addition, the importance of mγc residues Tyr-103, Cys-161, Cys-210, and Gly-211 was extended to IL-4 and IL-15 binding. Collectively, these data favor a model in which γc utilizes a common mechanism for its interactions with multiple cytokines.

EXPERIMENTAL PROCEDURES

Cloning of a Partial Rat γc cDNA—Reverse transcription-polymerase chain reaction (RT-PCR) was performed using the GeneAmp PCR kit (PerkinElmer Life Sciences) following the manufacturer’s recommendations. Briefly, 0.1–1 μg of rat spleen poly(A)⁺ RNA (CLONTECH Laboratories) was reverse transcribed for 15 min at 42 °C, in the presence of oligo(dT) primers. The cDNA was then amplified by AmpliTaq DNA polymerase, using two γc-specific primers (0.5 μM each). The primers corresponded to gene sequences of the mγc leader peptide (5'-AGATCTCTTCTAGTCCTNCAGC-3') and the rat γc cytoplasmic region (5'-CACCACTCCAGGCGAAGTCTCCC-3'). Amplification reactions were performed in a PerkinElmer DNA thermal cycler, starting with a 2-min denaturation at 95 °C, followed by 35 cycles of 30 s for melting at 95 °C and 45 s each for annealing/extension at 60 and 72 °C, respectively, and a final incubation of 7 min at 72 °C. The RT-PCR products were separated by 1% agarose gel electrophoresis, and the 800–900-bp major band was purified. The partial rat γc cDNA was subsequently digested with Sα1 and Xho1 and was used to replace a corresponding fragment of the mγc gene in the pSI-mγc vector. Plasmids containing the longer γc inserts (see "Results") were designated.
pSI-ratγc and encoded full-length γc protein with rat extracellular and transmembrane domains (residues 3–273) and the mouse carboxyl tail. Both the RT-PCR products and the rat γc inserts were analyzed by DNA sequencing.

Preparation of Chimeric Rat-Mouse γc—A conserved EcoRV site within the rat and mouse γc genes and a Xhol site in the pSI vector were used to digest the 5′ γc gene fragments from both the pSI-mγc and pSI-ratγc plasmids. These fragments, which encode the leader peptide and amino acids 1–99 of the γc were then swapped between the two vectors, resulting in γc cDNA that partially encodes rat and mouse sequences corresponding to the extracellular domains.

Expression Constructs and Maturation of Mouse γc—Full-length cDNAs for the mγc, human IL-2Rβ (hIL-2Rβ), and mouse IL-7Rα (mIL-7Rα) were inserted into the pSI mammalian expression vector (Promega, Madison, WI). The pDC032 expression vector containing the full-length mouse IL-4Rα (mIL-4Rα) cDNA was described previously (21) (provided by Immunex Corp., Seattle, WA). Site-directed mutagenesis of the mγc gene was performed by the QuikChange method (Stratagene, La Jolla, CA), as previously described (17). Mutations were verified by DNA sequencing.

Cell Culture and Transient Transfections—COS7 monkey kidney cells were maintained and transfected as previously described (17). All assays were done 3 days post-transfection, using COS7 cells harvested with 5 mM EDTA in phosphate-buffered saline.

Fluorescence-activated Cell Sorter (FACS) Analysis—Cytokine receptor surface expression in COS7 cells was analyzed as previously described using a FACScan flow cytometer (BD PharMingen) (17). The mAbs used were anti-mγc, 4G3 and 3E12, Ref. 21; TUGm2; human IL-7Rα, anti-IL-2Rβ (Mkβ3, BD PharMingen), or anti-IL-7Rα (ATR34; kindly provided by S. Nishikawa, Kyoto University, Japan) (23).

Radioligand Binding Assays—Recombinant human IL-2, IL-7, and IL-15 (PeproTech, Rocky Hill, NJ) and mouse IL-4 (Immunex, Seattle, WA) were radiolabeled with [3H] using IODO-GEN tubes (Pierce) to 15–60 μCi/μg. IL-2 and IL-7 binding was measured as previously described (17). IL-15 binding was measured on COS7 cells expressing hIL-2Rβ and mγc, as described for IL-2 γc-specific IL-2 and IL-15 binding was calculated as the cpm associated with hIL-2-wt/mγc transfected cells minus the cpm associated with control cells expressing hIL-2Rβ alone. γc-specific binding for IL-7 was calculated as the cpm associated with COS7-mIL-7Rα/mγc cells minus the cpm associated with COS7-mIL-7Rα cells. γc-specific ligand binding to COS7 cells bearing mutant receptors was expressed as percentage of the wild-type control. Nonspecific binding was always assessed by evaluating binding by mock transfected COS7 cells. This control was equivalent to that observed when blocking binding using an excess of unlabeled cytokine.

IL-4 Cross-Linking Assays—COS7 cells (4 × 10^5 cells) expressing mIL-4Rα in the presence or absence of mγc were incubated with 1 nM [125I]IL-4 for 2–3 h at 4 °C. The cells were then washed three times with phosphate-buffered saline and treated for 30–60 min at 4°C with 1 mM disuccinimidyl suberate in 1 ml of phosphate-buffered saline. After centrifugation, the cells were extracted with 200 μl of 0.5% Nonidet P-40 as described previously (24). The extracts were subjected to immunoprecipitation with 4G3 and 3E12 mAbs conjugated to Sepharose 4B beads at 4°C. Finally, the beads were washed three times with 0.5% Nonidet P-40, and bound radioactivity was measured in a γ-counter. In some experiments, the precipitated material was eluted with 1% SDS, separated by 10% reducing SDS-PAGE, and visualized by autoradiography (24).

RESULTS

Mapping the 4G3 and 3E12 Epitopes—The 4G3 and 3E12 mAbs to mγc recognize distinct epitopes (21), and their binding does not overlap with the epitope for TUGm2 (data not shown). 4G3 is a potent antagonist of IL-7, IL-9, and IL-15 bioactivity, whereas 3E12 is the most effective against IL-4 (21, 25). As 4G3 and 3E12 are of rat origin, the epitopes recognized by these mAbs should reflect differences in the three-dimensional structure between mouse and rat γc. Therefore, our strategy to define the epitopes to these two mAbs relied on comparing the amino acid sequences of mouse and rat γc. This first required determination of the sequence information of both the hIL-2Rβ and mγc cDNAs. Both appeared to be transcripts of the same gene; however, the shorter cDNA had a 97-bp deletion corresponding to putative exon 6 encoding the transmembrane region, suggesting that it was a splice variant of γc (Fig. 1A).

As anticipated, all defining features of the type I cytokine receptor superfamily were conserved between rat and mouse (Fig. 1B). The deduced amino acid sequences of the mature rat and mouse γc ectodomains (residues 1–241) were 88% identical to each other (Fig. 1B). The N-terminal 34 amino acids of γc were much less conserved (9 mismatches) than residues 35–241, which correspond to the cytokine receptor homology region (18 mismatches). It is also noteworthy that a lysine inserted into the mouse sequence at position 158 corresponds to a position previously identified to contribute to the binding of the TUGm2 mAb (17).

Because the number of mismatches between the rat and mouse γc ectodomain sequences was relatively large, we first assessed which segments of the mγc ectodomain contained the residues involved in the binding of mAbs by analyzing chimeric rat/mouse γc molecules. A conserved EcoRV site (Fig. 1A) was utilized for swapping cDNA fragments between the two species. After co-transfection of these chimeric cDNAs with the hIL-2Rβ into COS7 cells, the expressed proteins were examined for mAb and IL-2 binding (Table I). The latter assay was performed to ensure that the chimeric γc molecules were functional (Fig. 2). 4G3, 3E12, and TUGm2 mAbs readily stained cells transfected with wild-type mγc, but not wild-type rat or mouse (amino acids 1–99)/rat (amino acids 100–241) chimeric γc molecules. By contrast, rat (aa 1–99)/mouse (aa 100–241) γc chimeras fully retained their ability to bind 4G3, 3E12, and TUGm2. Thus, all three mAb epitopes were mapped to the membrane-proximal 141-amino acid segment of the mγc ectodomain.

Based on our past mutagenesis of mγc and that 5 of the 15 differences between positions 100 and 241 were conservative substitutions, six other mγc residues were replaced with their rat counterparts and then analyzed for their ability to bind mAbs to mγc after transfection into COS7 cells. The mutation V121E completely abrogated 4G3 binding, whereas staining with 3E12 and TUGm2 was unaffected (Fig. 2). Similarly, the R197L mutation dramatically reduced the binding of 3E12 but not the other mAbs to mγc. No other point mutations were found to inhibit the binding of 4G3 or 3E12. Thus, 4G3 likely recognizes an epitope near Val-121 in the first domain of mγc, whereas 3E12 may bind to a membrane-proximal part of domain 2. It is important to note, however, that whereas mutant V121E was defective in 4G3 binding, mutant V121A was not, suggesting that the side chain of Val-121 may not be directly involved in the mγc-4G3 interaction (Fig. 3A). Thus, this analysis may not have precisely defined the composition of these epitopes.

Point Mutations in mγc That Inhibit the Binding of IL-2, IL-7, and IL-15—We have previously established a ligand-receptor binding assay using transient transfected COS7 cells that distinguish mγc-dependent binding by IL-2 or IL-7 (17). Under our binding conditions (0.3–1 nm of radiolabeled ligand), readily measurable binding by IL-2 is strictly dependent upon expression of both the hIL-2Rβ and mγc subunits whereas mγc enhances the binding by mIL-7Rα. In the case of IL-2 binding,
Fig. 1. Sequence of rat γc. A, partial nucleotide and amino acid sequence determined from resulting cDNA amplified from rat spleen RNA by RT-PCR. The sequence was derived from a consensus of three independent clones and lacks only a segment corresponding to most of the cytoplasmic tail. Only amino acids of the mature rat γc are indicated. The 5′ PCR primer, the start of the rat sequence, a conserved EcoRV site, and predicted exon boundaries are marked. Exon 6, absent from a short form of rat γc, is underlined. B, amino acid sequence alignment of the mouse and rat γc extracellular domains. The putative TUGm2 epitope is boxed, and sites previously shown to be required for ligand binding by mγc are underlined. New mγc mutations are shaded in gray, and arrows indicate residues Val-121, Asn-128, and Arg-197. The rat γc sequence is found as GenBank™ accession number AF410926.

Because 4G3 efficiently blocks the detection of IL-7 and IL-2 cross-linked to mγc (21) and its epitope is membrane-distal, we hypothesized that mutagenesis of mγc near the 4G3 epitope may lead to the identification of novel contact residues involved in the binding of mγc-dependent cytokines. Using a structural model of γc as a guide (20), a number of amino acids in the vicinity of Val-121 were mutated to alanine. With the exception of the L124A mutant, which was undetectable, all the other mγc mutants were normally expressed on the cell surface (70% compared with the wild-type control) after co-transfection into COS7 cells with either hIL-2Rβ or mIL-7Rα. (Fig. 3, A and B). These same cells were also used in 125I-radiolabeled IL-2 or IL-7 binding assays (Fig. 3C). For all transfected COS7 cells that expressed cell surface mγc, there was a notable decrease (at least 2-fold) in mγc-dependent binding only for IL-7 when COS7 cells expressed mIL-7Rα and the mγc mutant N128A. Because the expression of hIL-2Rβ and mIL-7Rα was similar among these transfectants (data not shown), these results implicate Asn-128 in binding IL-7.

Mutations of Tyr-103, Cys-161, Cys-210, and Gly-211 of mγc have been previously shown to impair the binding of IL-2 and IL-7 (17). Therefore, we tested the contribution of these residues as well as those surrounding the 4G3 epitope for binding IL-15 (Fig. 4). Similar to IL-2, IL-15 also readily binds to hIL-2Rβ and mγc, but only in the presence of both subunits (8). Therefore, the same populations of transfected COS7 cells were examined for binding IL-2 or IL-15. This analysis indicated that COS7 cells expressing the Y103A or Y103R mutation of
mγc were impaired in binding both IL-2 and IL-15 (Fig. 4A). IL-15 binding was consistently more sensitive to the Y103R mutation than IL-2, perhaps due to different surface properties of the two cytokines. The C161S, C210S, and G211R mutant mγc molecules also did not support IL-15 binding (Fig. 4A). In contrast to the mutations at Tyr-103 and Asn-128 that do not affect mγc expression, these other three mutations typically resulted in lower mγc surface expression (Ref. 17; data not shown). However, based on our past work that established the relationship between expression of mγc as a function of IL-2 or IL-7 binding by their respective receptors (17), the virtually complete loss of IL-15 binding cannot be due to this suboptimal expression of mγc.

With respect to the mutations near the 4G3 epitope, only the N128A mutation somewhat inhibited IL-15 binding (Fig. 4B and data not shown). In these later experiments, COS7 cells were co-transfected with hIL-2Rβ and mIL-7Rα, and mγc to evaluate the effect of a given mγc mutation for IL-2, IL-7, or IL-15 binding on a single population of cells. To further assess the role of Tyr-103 and Asn-128 in binding these cytokines, mγc was mutagenized to contain both the Y103R and N128A mutations. After transfecting mγc containing either the individual or double mutations into COS7 cells with hIL-2Rβ and mIL-7Rα, ligand binding assays were performed (Fig. 4B). In this analysis, the binding of IL-2, IL-15, and IL-7 was decreased at least 2- to 3-fold by mutations at Tyr-103 whereas the N128A mutation caused a 2-fold decrease in the binding of IL-7 and a more modest decrease in IL-2 and IL-15 binding. However, the Y103R/N128A double mutation caused a 10-fold reduction in the binding of IL-2 and IL-7 and virtually abolished the binding of IL-15. Thus, these data indicate that both Tyr-103 and Asn-128 contribute to the binding of IL-2, IL-7, and IL-15, with Asn-128 playing a more critical role in the binding of IL-7. Collectively, our results indicate that IL-2, IL-7, and IL-15 exhibit similar requirements for Tyr-103, Asn-128, Cys-161, Cys-210, and Gly-211 in the putative mγc ligand-binding interface.

**Effect of Selected mγc Mutations on the Binding of IL-4—IL-4Rα/γc heterodimers have been reported to bind IL-4 with 3- to 5-fold higher affinity than IL-4Rα (3). However, in our hands COS7 cells expressing only mIL-4Rα always bound similar amounts of radiolabeled IL-4 as cells expressing both the mIL-4Rα and mγc subunits (data not shown). Therefore, we assessed the contribution of mγc to the binding of IL-4 by the capacity to cross-link radiolabeled IL-4 to mγc after its association to the IL-4R. The amount of radioactivity precipitated with anti-mγc was used to represent mγc-dependent IL-4R binding. This included both covalently cross-linked mγc-IL-4 complexes and IL-4 noncovalently associated to its receptor, as shown by the presence of two major bands after SDS-PAGE analysis of the immunoprecipitated material (Fig. 5A). This type of analysis revealed that the mγc mutations, K158A, which in part defines the epitope for TUGm2, and Δ(34–35) lacking amino acids 4–34 of the mature protein, readily binds IL-4. Therefore, as previously shown for mγc-dependent IL-2 and IL-7 binding (17), these regions of mγc are also not important for IL-4 binding.

All mγc mutations implicated in the binding of IL-2, IL-7, and IL-15 were also analyzed for their ability to contribute to the binding of IL-4. COS7 transfectants expressing mIL-4Rα and wild-type or mutant mγc were subjected to both IL-4 cross-linking assays and FACS analysis with anti-mγc. mIL-4Rα expression did not significantly vary among transfectants as verified by Western blot analysis (data not shown). However, our past analysis has shown that some of these mutations cause low mγc cell surface expression (17). This observation and the importance of mγc cell surface levels in this assay led us to first establish the relationship between the levels of cell surface wild-type mγc expression and the amount of 125I-IL-4 precipitated from extracts of cross-linked transfected COS7 cells. As expected, the fraction of mγc-dependent binding was proportional to the expression of mγc (Fig. 5B). These data served as a standard curve for the comparison of IL-4 binding by mγc mutants to similarly expressed wild-type mγc. The poorly expressed mγc mutants C161S, C210S, and G211R, bound 3- to 8-fold less IL-4 than similarly expressed wild-type (dotted line) or Q163A mγc (Fig. 5B). These results correspond
to the detection of minimal $^{125}$I-IL-4 for the C210A/G211A mutations when the precipitated material was analyzed on SDS-PAGE (Fig. 5A). Mutations at Y103A, Y103R, or N128A did not severely alter m$\gamma_c$ expression, as measured by staining with 4G3. Nevertheless, both mutations of Tyr-103 caused a partial (2- to 3-fold) decrease in the binding of IL-4, whereas N128A did not have a readily detectable effect when tested either alone or in combination with Y103R (Fig. 5B). These results suggest that Cys-161, Cys-210, Gly-211, and Tyr-103 are required for IL-4 binding, whereas Asn-128 is not necessary.

**DISCUSSION**

Our past work relied solely on molecular models of $\gamma_c$ to identify regions and amino acids within $\gamma_c$ that regulate ligand binding (17). In this report our strategy was to identify epitopes on m$\gamma_c$ for the inhibitory mAbs 4G3 and 3E12 as a means to further explore the structural basis of m$\gamma_c$-cytokine interactions. Localization of these epitopes was first dependent upon determining the sequence of rat $\gamma_c$ ectodomain. As expected, the rat- and mouse-deduced amino acid sequences were highly conserved. However, one unanticipated finding was that two forms of $\gamma_c$ cDNA were amplified by RT-PCR from rat spleen mRNA, one of which encodes a protein that lacks the sequence corresponding to exon 6 and is predicted to be secreted. These two forms likely represent products of alternative splicing. We have identified an identical spliced form of m$\gamma_c$ lacking the transmembrane domain for a variant of the mouse EL4 thymoma (Ref. 26). Although the overall biological relevance of this form of $\gamma_c$ remains to be determined, it is noteworthy that soluble $\gamma_c$ has been identified in human and mouse sera (27).

In this and a previous report (17), we have analyzed the ligand binding function of 33 mutant m$\gamma_c$ molecules harboring 39 distinct mutations. The extent and relative location of these mutations is displayed in Fig. 6A. Analysis of anti-m$\gamma_c$ mAb binding to these mutant molecules has revealed their epitopes (Fig. 6B). The 3E12 epitope is located in a membrane-proximal portion of m$\gamma_c$ near Arg-197 of domain 2. It is likely that 3E12 inhibits cytokine bioactivity by interfering with receptor-receptor interactions or by inducing a conformational shift in m$\gamma_c$ that indirectly affects receptor function. These two possibilities are not mutually exclusive. By contrast, the 4G3 epitope was localized to the vicinity of residue Val-121 in domain 1 because mutation of Val-121 of the m$\gamma_c$ to the corresponding rat residue (glutamate) abrogated 4G3 binding. However, Val-121 by itself does not define the 4G3 epitope as the V121A mutation did not interfere with 4G3 binding, and the surrounding sequences are almost identical between rat and mouse. Therefore, the 4G3 epitope likely depends on additional amino acids. One candidate region is located between amino acids 1 and 34 of m$\gamma_c$ because the deletion of this poorly conserved sequence, which is not required for IL-2, IL-4, and IL-7 binding, slightly inhibited the binding of 4G3 (17). The TUGm2 epitope, identified earlier (17), is centered around a unique lysine residue (Lys-158) of the m$\gamma_c$ sequence. The predicted locations of these epitopes and the inhibitory properties of the mAbs (3, 7, 21, 25, 28) suggest that it is highly likely that 4G3 and TUG2 antagonize $\gamma_c$-cytokine interactions by directly interfering with ligand-receptor interactions.

Based on this hypothesis, our search for new ligand-interacting sites was restricted to mutagenesis of selected residues of m$\gamma_c$ near the putative 4G3 epitope. This analysis led to the identification of amino acid Asn-128 in the linker region of m$\gamma_c$ that is necessary for the binding of IL-2, IL-7, and IL-15 but not IL-4. The N128A mutation caused a 2–3-fold reduction in m$\gamma_c$-dependent IL-7 binding while only modestly affecting the binding of IL-2 and IL-15. However, simultaneous mutation of Asn-128 and Tyr-103, a residue previously shown to contribute to IL-2 and IL-7 binding (17), essentially abrogated m$\gamma_c$-dependent IL-7 binding whereas the individual mutations had only a partial effect. This result clearly implicates both Asn-128 and Tyr-103 in m$\gamma_c$-dependent binding of all three of these cytokines. We cannot unambiguously distinguish by our assays whether this is an additive or synergistic effect.

The amino acids of the linker region, including Asn-128, are highly conserved in mammalian $\gamma_c$, consistent with an important role of this region in $\gamma_c$ structure and/or function. Although only one theoretical model of $\gamma_c$ (19) predicted a direct partici-
Of the large number of residues mutated and analyzed (Fig. 6A), relatively few have been implicated to participate in ligand binding (Fig. 6C). These include Cys-161 and Cys-210 that were proposed to form a putative intrachain disulfide bond (20), as well as Asn-128 and Tyr-103. It should be stressed that although mutations of these residues interfered with cytokine binding, they did not affect the binding of the conformationally sensitive 4G3 and 3E12 mAbs, indicating that the folding of mγc was not drastically changed (17). Importantly, most of these sites were required for the mγc-dependent binding of each of the four cytokines that we tested. The only exception was Asn-128, which did not obviously contribute to IL-4 binding, as mentioned above. Thus, our data favor a model in which γc-dependent cytokines use overlapping binding surfaces that include at least three loops (EF1, BC2, and FG2) of the mγc ectodomain (Fig. 6C). Tyr-103 is analogous to a structurally conserved aromatic amino acid present in several other members of the cytokine receptor superfamily (31) and which has been shown to directly participate in the binding of growth hormone and erythropoietin (32–34). Thus, Tyr-103 is an attractive candidate to directly participate in the binding of IL-2, IL-4, IL-7, and IL-15. Furthermore, Asn-128 is modeled to be in a position where it is exposed to solvent and also might directly interact with the cytokines. The ability of the Y103R/N128A double mutation to almost completely prevent IL-2, IL-7, and IL-15 binding is consistent with this view. The role of Cys-161 and Cys-210 in the binding of all four cytokines is less clear. As previously suggested (17), these two cysteines may maintain the conformation of the BC2 and FG2 loops, contributing to the local structure of the ligand binding interface rather than directly contacting the cytokines.

The IL-2R and IL-15R are also found as heterodimers as this permitted a direct evaluation of the effect of A134V mutation in human γc (30), as discussed above, raising the possibility that there may be other yet undefined contacts on γc for these cytokines. A more precise view of γc-cytokine interactions awaits solving the crystal structure of γc in the context of a heterodimeric receptor with bound ligand.

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