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Structural design and molecular evolution of a cytokine receptor superfamily

(hematopoietic system/interferon/tissue factor/fibronectin/immunoglobulin)

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ABSTRACT **A family of cytokine receptors comprising molecules specific for a diverse group of hematopoietic factors and growth hormones has been principally defined by a striking homology of binding domains. This work proposes that the ≈200-residue binding segment of the canonical cytokine receptor is composed of two discrete folding domains that share a significant sequence and structural resemblance. Analogous motifs are found in tandem ≈100-amino acid domains in the extracellular segments of a receptor family formed by the interferon-α/β and -γ receptors and tissue factor, a membrane tether for a coagulation protease. Domains from the receptor supergroup reveal clear evolutionary links to fibronectin type III structures, ≈90-amino acid modules that are typically found in cell surface molecules with adhesive functions. Predictive structural analysis of the shared receptor and fibronectin domains locates seven β-strands in conserved regions of the chain; these strands are modeled to fold into antiparallel β-sandwiches with a topology that is similar to immunoglobulin constant domains. These findings have strong implications for understanding the evolutionary emergence of an important class of regulatory molecules from primitive adhesive modules. In addition, the resulting double-barrel design of the receptors and the spatial clustering of conserved residues suggest a likely binding site for cytokine ligands.**

The ability of cytokines to influence the course of cell growth and differentiation uniquely depends on their recognition and binding by specific receptors; these cell surface molecules transduce the binding of messenger cytokines into cytoplasmic signals that trigger developmental processes within the cell (1). A model biological system that is distinctively controlled by a network of cytokine/receptor regulatory pairs is the hierarchical assembly of hematopoietic cells (1, 2). The sequences of known hematopoietic cytokines do not appear to be related (1); in contrast, the family of cognate receptors reveals a striking resemblance of binding domains (3–8). The extracellular segments of the interleukin (IL) 2, 3, 4, 6, and 7, granulocyte and granulocyte/macrophage colony-stimulating factor (G-CSF and GM-CSF), and erythropoietin (EPO) receptors share ≈200 amino acid modules that show a distinctive conservation of four cysteine residues in the N-terminal half and a "WSxWS" box (one-letter amino acid code; x is a nonconserved residue) near the C-terminal end (3–8). Similar motifs mark homologous domains in growth hormone (GRH) and prolactin (PRL) receptors (3).

A class of antiviral protein factors that also function within the hematopoietic network are the interferons (IFNs) (9). These cytokines are genetically divided into types I and II (IFN-α/β and -γ, respectively) and bind to distinct cellular receptors (9). Comparison of the protein sequences for the type I and II IFN receptors brings to light a common binding domain of ≈210 residues (albeit repeated in the type I structure) with characteristic cysteine pairs at both N and C termini (10). A third member of the IFN receptor family is tissue factor (TF), a membrane receptor for the coagulation protease factor VII (11). The "tether" function of TF in blood coagulation may signal the fortuitous recruitment of a mitogenic receptor by a wound healing response (11).

The superficial resemblance initially noted between hematopoietic and IFN receptors (10) portends a greater similarity in structure and internal symmetry. This work will show that the aforementioned receptors form a monophyletic superfamily with a distinctive architecture of duplicated domains within the ≈200-residue binding segments. In addition, the individual domains are distantly related to a common ≈90-amino acid structure known as a fibronectin (FBN) type III domain (12, 13). A predictive analysis of the generic domain fold in turn proposes that seven consensus β-strands form an antiparallel β-sandwich with a topology analogous to an immunoglobulin (Ig) constant domain. This model signals the potential discovery of a subclass of Ig-like proteins that have evolved from primitive adhesive modules to a present role in specific protein binding. Unlike antibody Ig domains, these receptor domains are predicted to rely on a very different binding paradigm.

METHODS

In comparing proteins that are very distantly or questionably related, sequence and structural pattern-matching methods are typically more sensitive than conventional algorithms in deriving structurally accurate alignments (14). In addition, patterns that incorporate both broad and specific sequence/structure information from a family of homologous proteins have a special predictive value in locating further homologs (3, 10, 11, 15). These techniques (16) simplified the task of compiling the multiple alignment of receptor domains.

Structural analyses of receptor and FBN domains used predictive algorithms (17) as well as mapping of amphipathic segments and β-turn regions to locate β-strands in the various protein chains (16). Consensus β-strand patterns defined the likely components of the core domain fold. This approach has been successful in modeling the secondary and tertiary structures of viral proteases (14, 16) and the α subunit of tryptophan synthase (18).

RESULTS

Shared Domains of Class 1 and 2 Receptors. The multiple alignment in Fig. 1*A* is the result of an extended process of sequence and structural template refinement. Sixteen se-

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Abbreviations: IL, interleukin; EPO, erythropoietin; G-CSF and GM-CSF, granulocyte and granulocyte/macrophage colony-stimulating factors; GRH, growth hormone; PRL, prolactin; IFN, interferon; TF, tissue factor; FBN, fibronectin; HEX, hexabrachion; LAR, leukocyte antigen-related protein.

quences are available from 10 different receptor types (3–8, 19–22). These sequences (and distinctive motifs; refs. 3–8) define the *class I* receptor family. In turn, the *class 2* receptor group is formed by the IFN receptors and TF (10, 11).

Alignments of class 1 and 2 receptors separately reveal 14 blocks of conserved sequences separated by variable, gapped linker regions. A core of identical or nearly identical residues across the various receptor sequences marks the conserved areas of chain; these residues usually form the nucleus of a predicted β-strand in each sequence block (Fig. 1*A*). This receptor secondary structure is characterized by an amphiphilic pattern of alternating hydrophobic/-philic residues.

Analogous patterns of sequence/structure conservation and variability in class 1 and 2 receptors suggested a natural superposition of alternating blocks and gaps. This resulted in a greater correspondence of similar residues within the 14 conserved blocks. Closer analysis by pattern-matching routines of the class 1 and 2 receptor alignment detected an internal duplication of an ≈100-amino acid domain containing 7 sequence (and β-strand) blocks (Fig. 1*A*). Notable conserved motifs include (*i*) a pair of proline residues that precede an amphiphilic β-strand in block 1, (*ii*) a conserved tryptophan in block 2 of the class 1 receptors that has spatial equivalents in the class 2 N-terminal (N) and class 1 C-terminal (C) domains, (*iii*) a characteristic pattern of aromatic residues separated by three residues in block 3, (*iv*) an alternating pattern of aliphatic and basic residues that follows a conserved tyrosine in block 6, and (*v*) the WSxWS box of class 1 C domains, which has fractured, degenerate equivalents in other block 7 sequences. Block 4 (and to a lesser extent, block 5) is a prominent segment of relatively nonconserved chain, both between sequences within a receptor class and between domains of both classes.

The superposition of class 1 and 2 modules suggests that the class-distinctive cysteine residues are dispensable for the *core* domain fold. However, the pattern of disulfide bonding between the scattered cysteines in either class 1 or 2 domains provides strong spatial constraints for tertiary folding. Chemical elucidation of disulfide bonds in the GRH receptor (*SS* in Fig. 1*A*; ref. 23) shows that bridges link residues in blocks 1 and 2, 4 and 5, and 6 and 7. The latter pair of GRH receptor disulfide bridges have class 2 equivalents.

The sequence similarity of repeated domains in class 1 and 2 receptors suggests an evolutionary relatedness. A further clue to this genetic homology is the striking similarity of gene structures for representative class 1 and 2 receptors. The exon junctions of the human GRH (25) and TF (26) genes map to equivalent loop locations in the extracellular protein chains: boundaries of the binding segment are defined by introns 1 and 5 (IVS1 and -5 in Fig. 1*A* precede N block 1 and follow C block 7, respectively; phase 1 introns*); the third intron (IVS3; phase 1 intron) marks the exact division of duplicated domains; introns 2 and 4 (IVS2 and -4; phase 2 and 0 introns, respectively) roughly halve the ≈100-residue domains and map to the central blocks of sequence variability.

A unique exception to the domain architecture of the class 1 and 2 receptors is the IL-7 receptor (7). While the C domain of the IL-7 receptor aligns easily with equivalent sections of the other class 1 proteins, the cysteine-rich N domain lacks significant similarity with any domain of the cytokine superfamily (and is excluded from the Fig. 1*A* alignment). Instead I suggest that the IL-7 receptor is a mosaic protein with an N domain that is related to a triplicated, cysteine-rich fold in the CD5 cell surface antigen (not shown) (31).

Structural Analysis of Shared Receptor and FBN Domains. Sequence patterns diagnostic of the conserved blocks of the ≈100-residue receptor modules detect a significant similarity to FBN type III domains in computer searches of protein databanks (15, 32).† In particular, the conserved block 2 tryptophan and block 6 tyrosine find spatial equivalents in typical FBN-like domains. These modular units have been identified in a wide variety of adhesive proteins (13) by homology to sequence motifs distilled from the 16 type III subunits of FBN (12). A sequence/structure analysis analogous to the one performed on receptor N and C domains was carried out with representative type III repeats from FBN (28), HEX (29), and LAR (33). Regions of chain equivalent to blocks 1–7 with corresponding β-strands could be readily identified (Fig. 1*B*). As was observed with receptor genes, FBN domains are exactly encoded by pairs of exons bounded by phase 1 introns and divided by a central intron that maps to a variable protein region (28).

The β-rich composition of receptor and FBN-like domains argues for a common, globular protein fold constructed from seven conserved β-strands. This prediction is in tentative agreement with available circular dichroic spectra for TF (34) and FBN (35). A likely folding motif for the domain β-strands is indicated by a survey of known all-β x-ray structures: these invariably form β-sandwiches of paired, amphiphilic β-sheets (24). However, several distinct ways of linking strands in β-sheet sandwiches have been described (24). The choice of strand topology for the receptor/FBN domain fold was partly guided by likely strand proximities dictated by disulfide bridges (Fig. 1*A*). In addition, domain sequence and structural patterns were systematically compared to analogous patterns from β-rich viral coat protein, tumor necrosis factor, β/γ-crystallin, plastocyanin/azurin, and Ig folds (24, 36). The "greek key" topology of the latter structures, by both of the above criteria, provides the best model for the canonical receptor/FBN domain fold (Fig. 1*C*).

Crystallographically analyzed Ig domains fall into two main structural classes that contain seven to nine β-strands (30, 36–38); the more economical constant domains serve as templates for the seven-stranded receptor/FBN domains. Fig. 2 *Upper* details the appropriate Ig-like topology of strands (*A–G* and *A′–G′* in N and C domains, respectively) decorated by residues that are universally conserved or characteristic only of class 1 or 2 receptors. The residues predicted to form the hydrophobic interior of the domain β-sandwiches are similar in character to analogous residues found in Ig folds (36, 38) but nevertheless suggest an altered sheet–sheet packing interface. (*i*) The receptor/FBN domains lack the characteristic intrachain disulfide bond that pins together the paired β-sheets, as well as a select tryptophan residue in strand *C* (38). Both of these features are noticeably absent from Ig constant-like domains found in varied cell surface molecules ("C2 set" or "H" domains; refs. 36 and 37). (*ii*) The hypervariable character of block 4 sequences in receptor/FBN domains, by analogy to Ig folds, is most likely explained by their structural role as exposed "edge" strands in the β-sandwich (38).

DISCUSSION

Model for Cytokine Binding. The structural analysis of receptor extracellular segments suggests the involvement of linked N and C Ig-like domains in forming specific cytokine binding sites. Fig. 2 *Lower* illustrates the likely disposition of domains connected by a hinge region of regular length with conserved prolines (C block 1; Fig. 1*A*). This double-barrel structure is similar to the crystallographically determined

*A phase 0 intron cleanly separates codons; phase 1 and 2 introns intercut a codon between the first and second or second and third bases, respectively.

†At the completion of this manuscript, a letter by Patthy (27) reported briefly on the sequence similarity (with no structural analysis) of FBN type III modules to only the C-terminal halves of a subgroup of class 1 cytokine receptors.

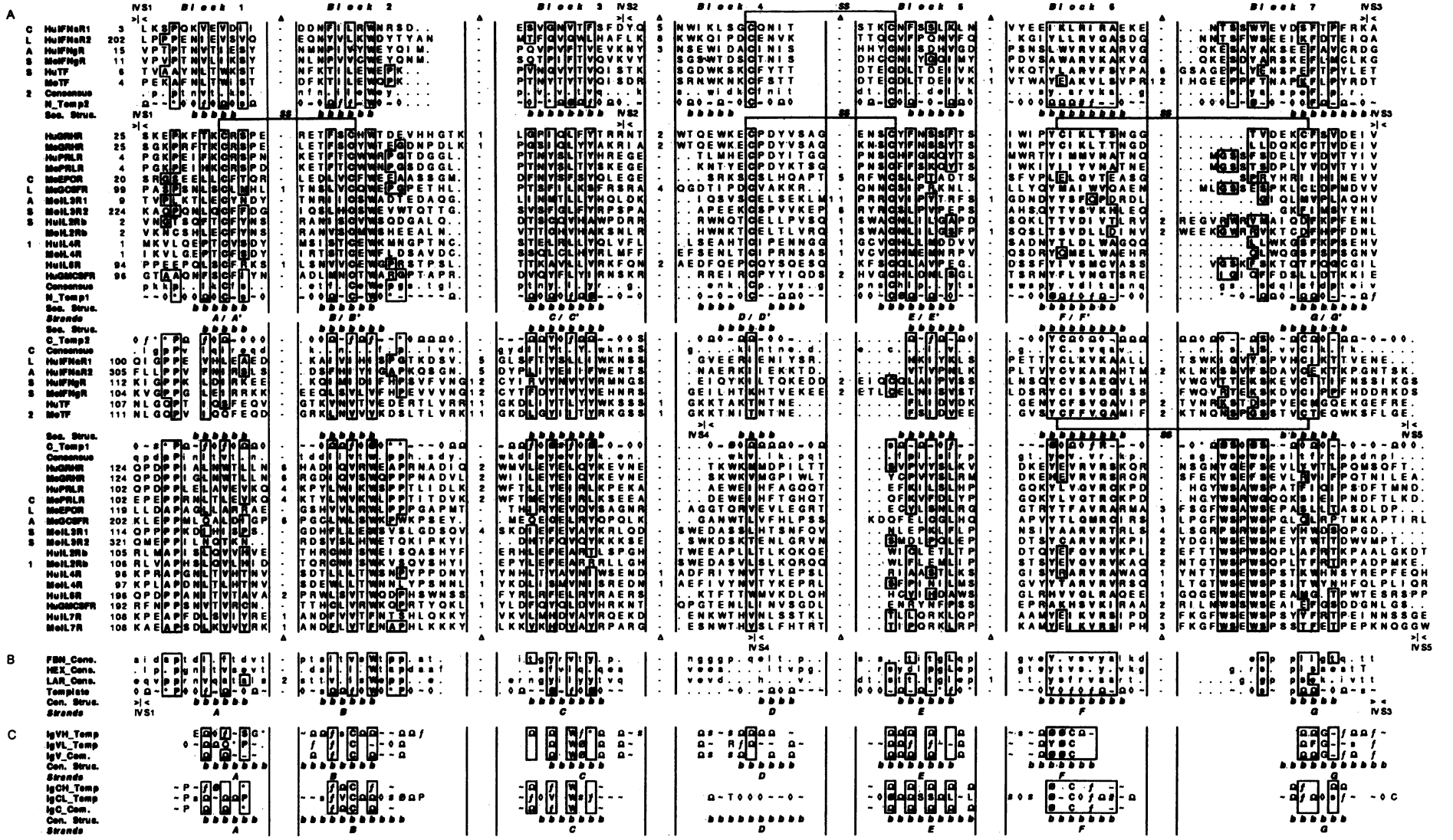

FIG. 1. (*A*) Sequence/structure alignment of duplicated domains in class 1 and 2 receptors. Human (Hu) and mouse (Mo) sequences of class 1 (3–8, 19–22) and class 2 (10, 11) receptors are aligned by component N (top tier) and C (bottom) domains. The duplicated segments of the IL-3 and IFN-α receptors are labeled R1 and R2; also, the IL-7 receptor contributes only a C domain. Each domain is similarly partitioned into seven blocks of conserved sequence separated by variable linker regions (Δ; residue interval noted). Within each block, amino acids that are conserved for a structural role in predicted β-strands (labeled *A–G* and *A′–G′*) or may help form the binding site are in bold type and boxed. Known (23) or predicted intrachain disulfide bridges (*SS*) are drawn between relevant cysteine residues. Under or over the multiple alignment, consensus lines list the most common amino acids at each position. Similarly, the template (Temp) line marks with symbols (Ω, hydrophobic; ◇, hydrophilic; Ø, aromatic; *f*, aliphatic; ~, small; ≈, large; #, charged; *, chain breaker; s, serine or threonine; residue composition of these sets following ref. 24) the common chemical and/or steric character of component residues. The position of introns mapped to the protein chains of GRH and TF receptors (25, 26) is noted by >|< symbols (where the junction intercuts or follows the codon under the first arrowhead) labeled IVS1 to -5. (*B*) Alignment and seven β-strands of consensus FBN-like sequences distilled from alignments

(*Legend continues at the bottom of the opposite page.*)

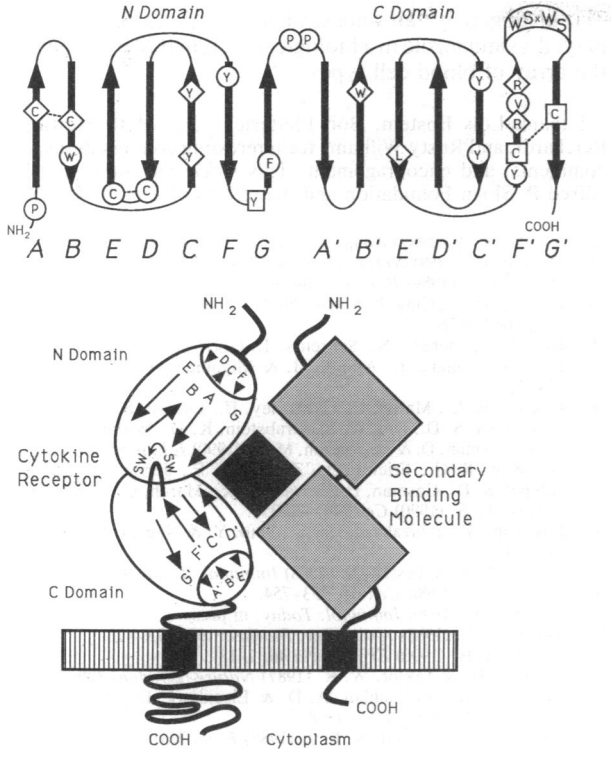

FIG. 2. The tertiary folding of β-strands in receptor domains. (*Upper*) β-Strand topology map of linked N and C domains drawn from the Fig. 1*A* alignment. Prominently conserved amino acids decorate the strands (arrows labeled as in Fig. 1*A*): circles mark residues peculiar to class 1 receptors, diamonds to class 2; square boxes encase globally conserved amino acids. The distinctive, class 1 WSxWS box is drawn in the *F'–G'* loop. (*Lower*) Predicted configuration of a canonical receptor binding segment on the cell surface. The linked domains pack with barrel axes at an angle (see PapD figure in ref. 38) so as to create a V-shaped trough lined by β-sheet surfaces; these converge on a hydrophobic hinge region with a proximal WSxWS loop. Linked to a transmembrane helix (black square) and a nonspecific cytoplasmic tail, the receptor is shown docked to a cytokine (gray diamond with receptor-recognition corner in black). In addition, the receptor/cytokine complex interacts with a secondary binding molecule that recognizes the free surface of bound cytokine as well as selected receptor loops distal from the pocket; in this case, the bilobal accessory molecule is analogous to the IL-2 receptor 55-kDa α chain (39).

fold of the *Escherichia coli* PapD chaperone protein (40). Holmgren and Branden (40) argued that the wide crevice between PapD Ig-like domains is a likely site for protein binding, and identified patches of hydrophobic and charged residues that are candidate interaction surfaces. Analogously, the prominently conserved residues that map to loops and β-sheet faces in both N and C domains may contribute to the formation of a generic cytokine cradle (Fig. 2 *Upper*), a skeletal framework that is enriched by additional class- and receptor-specific residues to facilitate the recognition and binding of cognate cytokines.

The observation of homology between receptors has revitalized the view that a number of hematopoietic factors, growth hormones, and IFNs may exhibit a parallel convergence of structure that is not evident in amino acid chain comparisons: perhaps helix-rich cytokines have similar antiparallel helix-bundle folds (refs. 3, 7, and 10 and references therein). Receptor-binding epitopes of cytokines with known tertiary folds typically map to the exposed surface of a particular bundle helix (10). The structural nature and likely dimensions of the proposed binding site in receptors suggest that helical cytokines "wedge" sideways and preferentially dock a "corner" structure (i.e., the receptor-recognizing helix) into the V-shaped crevice between linked Ig-like domains (Fig. 2 *Lower*). The conserved WSxWS box, predicted to lie on a loop between C-domain strands *F'* and *G'*, helps form the floor of the binding crevice. This model also suggests that accessory binding molecules are free to contact another face of the bound cytokine, as well as potentially interact with receptor loops distal from the binding crevice.

Evolutionary Implications. X-ray crystallography has shown that the tandem duplication of protein motifs is a common design strategy for enzymes and binding proteins; this internal symmetry is most often cloaked by the divergence of amino acid chains (41). In special cases, clues to the evolutionary origin of repetitive motifs are revealed by homology to more primitive, single-domain proteins (e.g., see ref. 14). Analogously, the duplicated domains of cytokine receptors have evolutionary relatives in FBN-like subunits and, as proposed, distant structural ties to primitive Ig modules.

As demonstrated by Cohen *et al.* (42) for Thy-1 antigen, the comparative register of predicted and determined β-strands is persuasive corroborating evidence for the structural kinship of weakly similar sequences with Ig folds. The Ig link to receptor/FBN domains has analogous roots in the comparative analysis of sequence and structural patterns (14–16, 18, 36); these result in the proposal of a minimal Ig-like framework structure for each domain (Fig. 2 *Upper*). A novel aspect of the consensus domain fold is a meager identity with minimal sequence patterns diagnostic of variable or constant (C1 set) domains, or with motifs derived from the economical (C2 set) domains of cell surface Ig-like molecules (30, 37). However, a number of Ig candidate sequences lack these amino acid descriptors (36); in addition, an excess of cysteines in surface antigen molecules such as CD5 or Ly-6 (as in the class 1 N domains; Fig. 1*A*) confounds their Ig classification (37). The structural homology of *E. coli* PapD to Ig constant domains is not accompanied by an equal measure of sequence identity; indeed, a new "C3 set" of Ig structures is proposed to contain the bacterial protein and a surprising mammalian homolog, CD5 (40). The predicted ties of receptor and FBN domains to Ig may only indicate a structural convergence to a stable β-rich fold; still, the functional similarities with Ig-like PapD suggest a tentative classification of receptor/FBN domains in a very distantly related subgroup of the Ig superfamily.

The sophisticated function of high-affinity protein binding exhibited by cytokine receptors has more in common with antibodies than primitive Ig-like molecules with nonspecific adhesive properties. However, the proposed model for cytokine recognition (and the PapD mechanism) illustrates a binding paradigm unlike that utilized by antibody domains. Paired variable folds utilize genetically variable loops to form combining sites for diverse antigens (30, 37, 38); in contrast, cytokine receptors are predicted to use a fundamentally less flexible strategy by adapting the trough between linked β-sandwiches to serve as a ligand binding site (Fig. 2 *Lower*). The constrained topography of the trough is a strong discriminant for ligand recognition; in addition, the structural role of trough-lining residues in β-sheet conformation is a hindrance to change by genetic variation. As previously discussed, a strong clue to a preferred ligand "shape" comes

FIG. 1. of multiple domains of FBN (27), hexabrachion (HEX; ref. 28), and leukocyte antigen-related protein (LAR; ref. 29). Intron positions are noted only for IVS1 and -3; IVS2 maps to variable locations in block 4. (*C*) Sequence templates for β-strands of variable (V) and constant (C) Ig domains (with heavy- or light-chain variants; H or L) are drawn from refs. 24 and 30. These restricted chain segments are aligned with blocks of receptor/FBN domains to show residue similarities in β-strands *A–G*.

from the convergent x-ray and predicted helical folds of diverse cytokines (3, 7, 10). This may indicate that the predecessor of both class 1 and 2 receptors had an unremarkable but specific affinity for a helix-bundle protein (akin to the specialization of FBN domains in divergently binding DNA, heparin, or fibrinogen; refs. 12 and 28); importantly, this trait has been "frozen" into the structural makeup of descendant receptors (Fig. 3). The functional degeneracy of class 1 and 2 cytokines (characterized as pleiotropic factors with a broad spectrum of activities; refs. 1 and 2) may have a possible structural basis in a promiscuous binding strategy of homologous receptors that dock ligands of similar folds (3).

Several Ig-superfamily members have apparently converged on a similar plan for binding mitogenic proteins: the platelet-derived growth factor/macrophage colony-stimulating factor receptors (43) and the IL-1/fibroblast growth factor receptors (44) have binding segments that are constructed of Ig repeats. Surplus (C2 set) Ig domains in IL-6 and G-CSF receptors (3, 8) and the swapping of a receptor N domain for a CD5-like (C3 set) Ig module by the IL-7 receptor (7) may illustrate an evolutionarily recent intermixing of structurally compatible molecular building-blocks. This latter mechanism, added to the potential shuffling of segments encoding entire or half domains between receptor genes, may represent plausible strategies for the diversification of receptors and the acquisition of new affinities by structurally constrained binding pockets.

The detection of repetitive motifs within class 1 and 2 molecules has implications for tracing the molecular emergence of a receptor superfamily from a class of primitive adhesive modules (Fig. 3). The presence of FBN components in growth-regulating molecules has other parallels: these modules are commonly found in diverse cell surface proteins (often paired with Ig domains) that influence neural development in organisms (13). However, these latter molecules do not appear to direct biological change by binding protein factors but rather act as pattern-forming determinants in guiding cell–cell interactions (13). This kind of morphoregulatory role is considered a more primitive function of FBN and Ig progenitors (13, 30, 37); analogously, an ancestral receptor molecule may have played a similar role in a biological system that evolved to form the array of blood cell types.

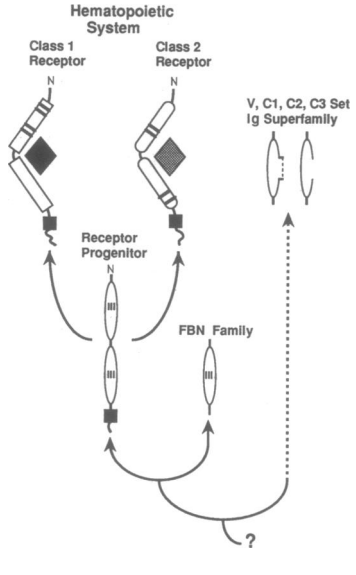

FIG. 3. The evolutionary emergence of cytokine receptors from primitive adhesion molecules. Class 1 and 2 receptors (drawn from refs. 3 and 10; altered to show duplicated structural nature and V-shaped binding pocket for diamond-shaped cytokines; dark bands are distinctive cysteine residues) diverge from a common ancestral receptor composed of two linked FBN-like domains. In turn, there are clear evolutionary links to other FBN-like molecules, as well as a more tentative identification of receptor/FBN domains with the Ig superfamily.

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