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Molecular model of interleukin 12 that highlights amino acid sequence homologies with adhesion domains and gastrointestinal peptides

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A three-dimensional (3-D) model of both subunits of interleukin 12 (IL-12) has been created through molecular modeling. Initial assignment of coordinates in the model of the p40 subunit was based on established amino acid sequence homology between the second and third domains of p40 and the human growth hormone receptor (GHR) and new observations of similarity between the first domain of p40 and the N-terminal domain of CD4. Human growth hormone (GH) served as the reference protein for the p35 chain. Furthermore, thorough analysis of the amino acid sequence of IL-12 revealed two distinct regions of the p40 subunit that display homology with other proteins. The first region (in domain two) contains the sequence RGD, which is found in adhesion proteins (such as fibronectin), and the nearby sequence VTCG, which occurs in a diverse set of molecules, including thrombospondin, properdin, and circumsporozoite proteins of Plasmodium. *The second region of homology spans the third domain of p40 and shows marked similarity with the gastrointestinal peptides, such as secretin and glucagon and their preprohormones. We conclude (1) that the regions of homology define functionally important segments of p40 that are fully exposed at the protein surface, and (2) that the third domain of p40 (and its equivalent in the cytokine receptor family) is derived from the same ancestral genes as the gastrointestinal peptides.*

Keywords: interleukin 12, molecular modeling, adhesion sequences, gastrointestinal peptides

Color Plates for this article are on pages 143–144.

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Received 23 January 1996; accepted 16 May 1996.

INTRODUCTION

The cytokine, interleukin 12 (IL-12), which was formerly known as natural killer cell stimulatory factor[1] or cytotoxic lymphocyte maturation factor,[2] is emerging as an important subject for clinical immunology. Originally, IL-12 was identified on the basis of its ability to induce interferon γ (IFN-γ), enhance the growth and cytolytic activity of natural killer (NK) cells, and promote the T cell response to mitogens.[1] Subsequently, it was discovered that this factor also synergizes with IL-2 in the expansion of T cells and lymphokine-activated killer cells, potentiates the cytolytic function of T cells, and influences the balance of T_h1 versus T_h2 immune responses (reviewed in Ref. 3). It also restores cell-mediated responses of T cells from human immunodeficiency virus (HIV)-infected individuals.[4] In addition to these effects on cultured lymphocytes, IL-12 provides important immune regulation *in vivo*. Thus, it has been shown that administration of IL-12 to susceptible mouse strains can prevent fatal infection by *Leishmania*.[5,6] Infection of mice by other parasites, including *Toxoplasma gondii*[7] and *Plasmodium chabaudi*,[8] is also effectively controlled by IL-12. It appears that the protective effect is accomplished mainly by the induction of cellular responses mediated in part through the production of IFN-γ. Finally, in an animal model of tumor development, daily injection of mice with IL-12 diminished tumor growth and enhanced survival rates consistent with an effect on cell-mediated immunity.[9]

Normally, IL-12 is synthesized by B cells, monocytes/macrophages, and dendritic cells as well as certain other specialized antigen-presenting cells.[10] The active molecule is composed of two subunits with molecular masses of 35 kDa (p35) and 40 kDa (p40) that are disulfide bonded to form the active complex.[3] Interestingly, the p40 subunit is produced in vast excess of the p35 chain and may be secreted as a p40 homodimer.[2] The physiological significance

Journal of Molecular Graphics 14:148–157, 1996

655 Avenue of the Americas, New York, NY 10010

S0263-7855/96/$15.00
PII S0263-7855(96)00044-6

of this asymmetrical production of IL-12 subunits is unclear.

The genes for both IL-12 subunits have been cloned and characterized.[11,12] Analysis of the amino acid sequences revealed homology between p35 and other cytokines such as IL-6 and granulocyte colony-stimulating factor (G-CSF),[13] and surprisingly, a significant homology between p40 and receptors for cytokines and growth factors.[14] This set of cytokine receptors includes those specific for IL-6, IL-3, IL-4, IL-7, prolactin, erythropoietin, growth hormone, and several others.[15,16] Thus, it appears that IL-12 is a chimeric molecule having both ligand and receptor-like domains. However, to date there is no detailed information concerning the structure of this important cytokine.

In an effort to elucidate the major structural features of the IL-12 molecule, a three-dimensional (3-D) model has been constructed on the basis of homologies with proteins whose crystal structures have already been solved. The starting point for the computer modeling was the human growth hormone receptor (GHR), which is similar to the second and third domains of the p40 subunit. The first domain of p40 was modeled on the basis of homology with the CD4 protein, while the p35 subunit was modeled after growth hormone (GH) as reported here. During the analysis of the amino acid sequence of IL-12, we have identified additional noteworthy regions of p40. These regions bear striking similarity to adhesion sequences from proteins such as fibronectin and properdin and with the gastrointestinal proteins (e.g., glucagon and secretin). The molecular model of IL-12 reveals the predicted topological arrangement of these regions of interest.

METHODS

Amino acid sequence homologies

The original amino acid sequence similarities between p40 and the IL-6 receptor (IL-6R) have been noted previously,[14] as have the similarities between the IL-6R and GHR.[15,16] The same alignment of the IL-6R and GHR (with minor modifications) was used to produce the comparison between p40 and GHR, which is shown in Figure 1. The similarities between p40 and CD4 and the presence of the RGD and VTCG sequences in p40 were revealed by close visual inspection of the amino acid sequence of IL-12. Precise sequence matching was determined using the alignment function in the Homology software module from Biosym Technologies (San Diego, CA). Using the manual setting, segments of the proteins were analyzed for the highest degree of homology according to the Dayhoff et al. convention of identity or conservative replacements among amino acids (mutation scoring matrix).[17] The amino acid residues have been numbered according to their positions in the mature IL-12 protein. The sequence information has been presented here in the single-letter code as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

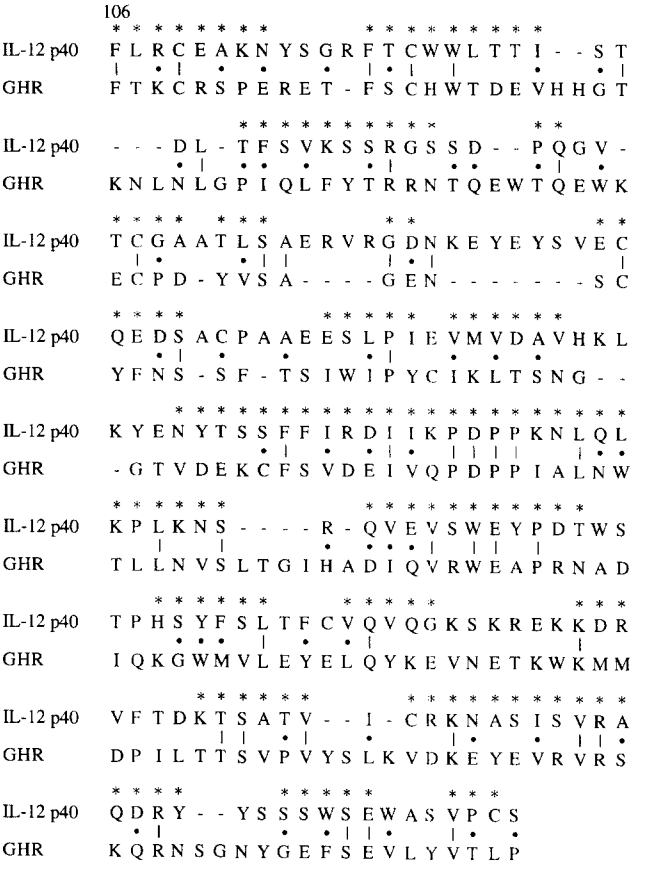

Figure 1. Alignment of D2 and D3 of the p40 subunit of IL-12 and the human GHR. The single-letter code has been used to denote the amino acid residues. A vertical line between the sequences indicates identity at these residues; a dot signifies a conservative amino acid change. Asterisks above the sequence mark those segments considered to be SCRs.

Molecular modeling

Molecular modeling studies were performed on a Silicon Graphics Indigo 2 computer outfitted with software from Biosym Technologies. Model building was performed with the Homology module in Insight II and energy minimization of the structures was accomplished with the Discover module. Construction of a plausible 3-D model by Homology requires identification of a suitable reference protein whose known structure serves as a framework for assigning atomic coordinates to the homologous protein. To test whether the model would be reasonable, secondary structure predictions for IL-12 were obtained using the Chou and Fasman[18] and GOR II (Garnier and Robson)[19] algorithms provided with the software and were compared with the structure of the reference protein. The solved protein structures and loop templates were accessed from the Protein Data Bank (PDB)[20,21] (release 70, October 1994). The second and third domains of p40 (amino acid residues 106–214 and 215–306, respectively) were modeled on the basis of sequence homology with the human GHR (PDB entry, 3hhr).[22] Amino acid sequence analysis of the first domain (D1) of p40 revealed similarities to the first domain of human CD4. Thus, assign-

ment of backbone coordinates in D1 was based on the solved structure of the CD4 protein (PDB entry, 3cd4).[23] p35 shares amino acid sequence homology with cytokines such as IL-6 whose structure is not known. However, there are also similarities to human GH and in view of the fact that p40 was modeled after the GHR, it seemed a logical choice to employ GH (PDB entry, 3hhr) as the reference protein for p35.

In building the model, structurally conserved regions (SCRs) were assigned first on the basis of homology and topological considerations. Areas displaying the greatest degree of homology were identified by a combination of visual inspection of the sequences and use of the manual alignment and mutation scoring matrix of the Homology software. A desire to conserve the overall strand arrangement of the reference protein imposed an additional constraint on the choice of SCRs in the model protein. Before the protein alignments were finalized, the initial positioning of the cysteine residues involved in disulfide bonding was evaluated to determine whether the proximity was adequate for pairing.

Generally, the reference protein and the domain to be modeled were similar in length. However, gaps were introduced into the sequences to optimize homology or to better define the SCR. Frequently, the gaps occurred in loop regions such that altering the size or orientation of the selected loop could easily accommodate the differences from the reference protein. In other cases, the gaps occurred in the alternating strands and the splice points required repair during energy minimization runs.

After atomic coordinates were assigned to the SCRs, it was necessary to build loops that connected the fixed regions. Loop search is performed by comparing the C_α distance matrix of the SCRs bordering the loop region with a precalculated matrix for all known proteins that have loops spanning a similar gap and with similar-sized flanking regions. The top choices for the loops are those with the best overall values for the root-mean-square (r.m.s.) distances between the database proteins and the modeled structure over the flanking regions. During loop search, the top 30 computer-generated choices were examined in detail to determine the best fit. The loop search covered only those Brookhaven pdb files of structures resolved to 2.0 Å or less in order to ensure the greatest fidelity of the modeled loops. Careful examination of the 3-D structure of the loop region was achieved using the stereo mode and CrystalEyes electrostereographic system (StereoGraphics Corp., San Raphael, CA). The two major criteria for selecting the top choice were proximity of the splice points at fixed atoms and accommodation of the amino acid side chains into the available space.

Once the atomic coordinates of the loops were assigned, the van der Waals overlaps between the atoms in the model were identified (distances <0.7 Å) and fixed, typically by minor rotations of the side chains. Splice points were then repaired and energy minimization of the structure (300 iterations) was performed in the Discover module.

The predicted structure of domain 1 (D1) derived from CD4 begins at residue 9. Using the Biopolymer module of the Insight software, an extended peptide segment encompassing the N-terminal eight residues was built. The first three residues were arranged as a turn, in keeping with secondary structure predictions. This peptide segment was then bonded to D1 at residue 9 and rotated to extend this strand logically while minimizing steric overlaps. The modified D1 was subjected once more to an energy minimization run to refine the structure. The arrangement of the first four N-terminal residues of p35 was determined in the same manner. To arrive at the final structure of IL-12, the model of D1 was grafted onto the structure of D2–D3. This was accomplished by carefully aligning the equivalent G–A strand that bridges the domains and then rotating D1 about this axis while minimizing steric interference between the side chains. Only one global orientation of D1 provided a superior fit. As before, van der Waals overlaps were identified and corrected. Generally models have been depicted here as either ribbon structures or as CPK renderings using the Biosym software (Insight II).

RESULTS

To generate a plausible 3-D structure of a protein through computer modeling, there must be a defined amino acid sequence homology with proteins whose structures have previously been solved by X-ray crystallography or nuclear magnetic resonance (NMR). It has already been reported that the p40 subunit of IL-12 is strikingly similar in sequence to the class 1 cytokine receptor family, which includes the GHR.[14] However, because direct comparisons between the sequences of p40 and GHR have not previously been published, they have been shown here in Figure 1. This alignment was used to create the molecular model described in this article. The original report of homology between IL-12 (p40) and the cytokine receptors focused on similarities with the IL-6R, whose structure is not known. However, we have observed additional sequence homology between p40 (D1 in particular) and CD4, a surface receptor of helper T cells (Figure 2). Over their N-terminal domains, p40 and CD4 share 23 identical amino acid residues in common (23% identity, 39% similarity). This is exactly the same number of identical residues shared between p40 and the

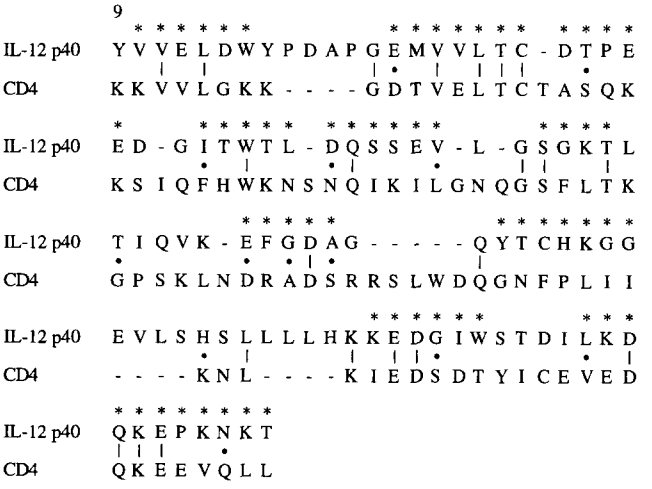

Figure 2. Sequence alignment of p40 with the first domain of human CD4. The symbols used here have the same meaning as in Figure 1. The CD4 sequence is from Ref. 23.

IL-6R over the same stretch of sequence. Consequently, CD4 would appear to be a desirable starting point for modeling D1 of p40 given that the high-resolution structure of CD4 is available. Figure 2 depicts the precise alignment used for predicting the 3-D structure of the p40 subunit.

The p35 subunit has been reported to be homologous to the cytokines IL-6 and G-CSF.[13] Our own analysis revealed additional similarities to IL-4 (an α-helix bundle structure) and growth hormone (GH). Secondary structure predictions based on the sequence of p35 confirm a preponderance of α-helical structure (data not shown). Because p40 is similar to the GHR, human GH was chosen as the reference protein for model building. The protein alignment used for generating the model is shown in Figure 3. With slight adjustments along this sequence there is 22% identity and 47% similarity between these proteins at the amino acid level.

Assignment of the initial atomic coordinates was based on the homologous regions of the proteins defined above. A major constraint imposed during model building was the alignment of the cysteine residues involved in both intrachain and interchain disulfide pairing, in particular, between residue C74 in p35 and C177 in p40.[24] In addition, the goal was to preserve the strands of β-sheet structure, where possible, and either to enlarge or shrink the loop regions to accommodate gaps in the sequence alignments. The predicted structure of p40 is shown as a stereo diagram in Color Plate 1 and has been represented as a solid ribbon (D1 in green, D2 in orange, and D3 in gray). Together, D1 and D2 comprise a globular region at the top of the molecule while the third domain forms a stem or stalk at the bottom. Various regions of interest on p40 (which are discussed below) have been highlighted in different colors. The arrangement of the p35 subunit in the model is also shown (at the left in Color Plate 1, in blue). The locations of the cysteine residues that form intrachain disulfide bonds have been highlighted in red. Each of the three domains of p40 provide contacts that stabilize the association with the p35 subunit. Overall, the 3-D shape of IL-12 resembles a mushroom with three of the four domains at the top potentially available for interaction with the IL-12 receptor.

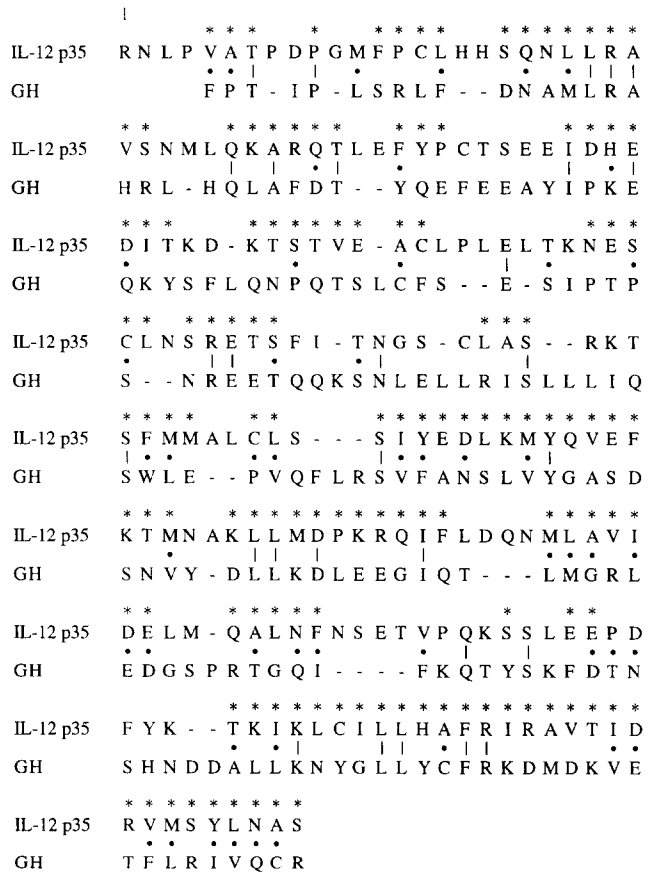

Figure 3. Sequence alignment of p35 and human GH used for molecular modeling. The alignment depicted here underrepresents the homology between the molecules for the sake of preserving overall structural integrity.

During the analysis of the amino acid sequence of the p40 subunit to build the structural model, it was noticed that two regions were particularly interesting (depicted in Figure 4a–c[11,26–35]). First, human p40 contains an RGD sequence in the middle of D2 (residues 159–161), which is a hallmark feature of adhesion molecules, such as fibronectin, that bind to members of the integrin family of receptors.[36] The cytokine receptor family bears homology to the type III domains of fibronectin[37]; however, the presence of the RGD sequence in p40 has not been emphasized before. Although murine p40 does not contain RGD, it does include the sequence QEDV in the same vicinity, which is similar to some of the alternative integrin ligands[38] displayed in Figure 4a. Interestingly, further analysis of this region of D2 revealed the sequence VTCG and flanking regions that are identical to the adhesion sequences observed in thrombospondin, circumsporozoite proteins of *Plasmodium*, and is similar to the corresponding sequence in properdin (Figure 4b).[32,33] Thus, it appears that the RGD/VTCG portion of the p40 bears a striking resemblance to the active sites of adhesion molecules that bind to integrins.

The second region of interest revealed similarities between D3 of p40 (residues 263–306) and members of the gastrointestinal peptide family of hormones (Figure 4c).[34] As shown in the Figure, the N-terminal portion of this segment from p40 is very similar to the N-terminal portion of peptides like VIP, PHM-27 and secretin. The central region of this segment is more similar to the central portion of the neurohormones, peptide YY and neuropeptide Y,[35] however this part of p40 also has the sequence SVRAQD which is nearly identical to the sequence in glucagon, SRRAQD (although this region was not aligned in the Figure shown here to avoid undue insertion of gaps). The C-terminal end

Figure 4. Sequence homology between the p40 subunit of IL-12 and adhesion domains and gastrointestinal peptides. Segments of p40 have been aligned with discrete regions of proteins mediating cell adhesion (a and b) or with members of the glucagon-secretin family and certain neurohormones (c). The single-letter code for amino acids has been used here. Identical residues have been outlined whereas conservative changes have been represented with a different font and highlighting. The protein sources have been listed clearly and the residues have been numbered at the right. Sequences were obtained from the following sources: IL-12,[11] laminin A chain,[26] vitronectin,[27] C3 of complement,[28] fibronectin,[29] murine IL-12,[30] fibrinogen γ chain,[31] and the thrombospondin, properdin, and Plasmodium *proteins.[32,33] Abbreviations in (c): GRF, growth hormone releasing factor; PHM-27 (same); Gluc-like, glucagon-like peptide; ACAP, adenylate cyclase-activating polypeptide; Secretin (same); PHV (same); GIP, gastric inhibitory peptide; VIP, vasoactive intestinal peptide; Glucagon (same); Pre, prealbumin; Neuro Y, neuropeptide Y; Peptide YY (same), BPP, bovine pancreatic polypeptide. The dots above the sequence in (c) represent highly conserved amino acids (≥50% of the members with that residue or a similar counterpart). The sequences of the gastrointestinal and neural peptides were derived from previous work by Jörnvall* et al.*[34] and Tatemoto.[35]*

of this p40 segment is homologous to both the carboxy-ends of the GRF and PHM-27 peptides and to the more central region of the neurohormones.

The topological arrangement of the various regions of segmental homology has been highlighted in Color Plate 1. The RGD sequence (in purple) is clearly exposed at the protein surface on a face of p40 that may contact the IL-12 receptor. The VTCG adhesion sequence also lies in this vicinity (represented in magenta) and is largely exposed to solvents, although the cysteine residue in this segment forms a disulfide bond with the cysteine at position 171 and is buried within the structure. The two most prominent areas of homology with the gastrointestinal peptides are found on exposed loops in D3 in the stem region of p40 (depicted in yellow). The sequence RYYSS appears to contribute important stabilizing contacts with the p35 chain. On the other hand, the homologous region defined by the sequence VFTD is rotated approximately 45° away from the first segment and would appear to provide only minor contacts to the N-terminus of p35.

The observation of sequence similarities between p40 and the gastrointestinal hormones led us to examine the sequences in greater detail for clues about their possible meaning. Because hormones like glucagon and secretin are derived by proteolytic processing of larger precursor proteins, the amino acid sequences of the secretin[39] and gluca-

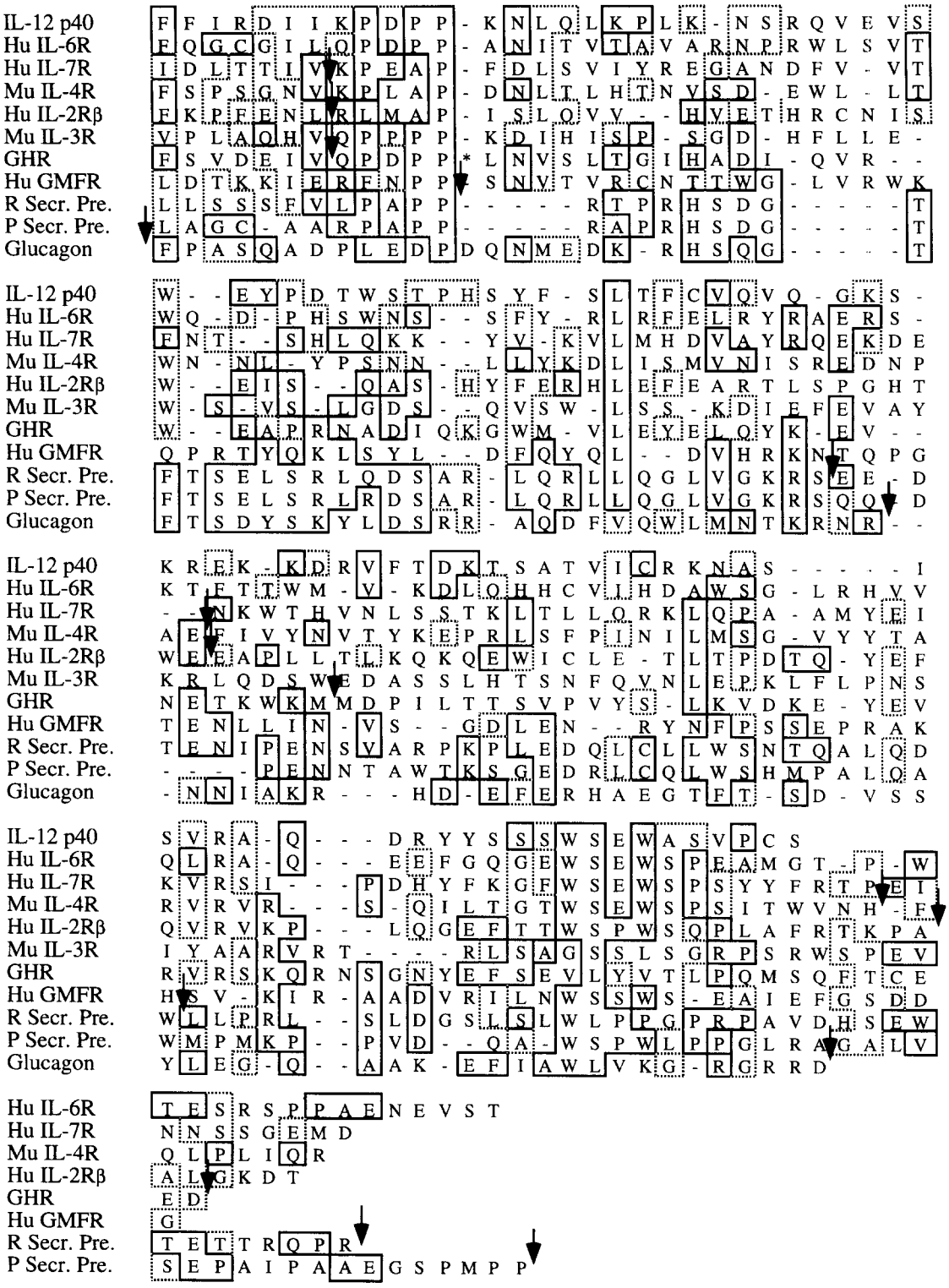

Figure 5. Alignment of the second domains of the cytokine receptor family, including IL-12 p40, the precursor protein for secretin from rat (R) and pig (P), and glucagon. The cytokine receptor sequences and gap insertions are from Cosman et al.[16] *with a few modifications. Identical residues are boxed by a solid line, whereas conservative changes are denoted by a dotted line comprising the box. Hu GMFR, human granulocyte-macrophage colony-stimulating factor receptor; the other abbreviations are self-explanatory. The common arrangement of intron/exon junctions among members of the cytokine receptor family and gastrointestinal preprohormones has also been shown. The protein sequences and exon junctions are based on published reports: IL-4R,[41] IL-2Rβ,[42] GHR,[43] secretin,[39] and glucagon.[40] Arrows above the sequences indicate intron/exon junctions.*

gon[40] precursors were examined for evidence of further similarities with this region of p40 and other members of the cytokine receptor family. The results of this analysis are presented in Figure 5.[16,39–43] Clearly, there is marked sequence homology between secretin & glucagon and the second/third domain of various cytokine receptors, including p40. Perhaps significantly, a potential proteolytic processing site is just upstream (3 residues away) from the segment of p40 depicted in Figure 4c. The closest resemblance among the proteins shown here is observed between secretin and the human IL-2 receptor β chain which share an overall similarity of about 45% (based on conservative amino acid substitutions). Over the stretch depicted here, rat secretin shared 20 identical residues with IL-2Rβ while porcine secretin shared 22 residues. This compares favorably with the degree of identity between IL-2Rβ and the human receptors for IL-6 and IL-7 which share 21 and 17 identical residues, respectively, over this same stretch of the proteins. Moreover, both rat and porcine secretin have 20 identical amino acids in common with the IL-4 receptor which represents greater homology than that observed between the murine IL-4 receptor and the murine IL-3 receptor (14 identities) or between IL-2Rβ and the human receptor for granulocyte-macrophage colony-stimulating factor (12 identities). A similar number of gaps are found in all of the amino acid sequences depicted here, including the secretin proteins, indicating that these direct comparisons are valid. To complete the amino acid sequence comparisons, the p40 subunit of human IL-12 shares 70% identity with its murine counterpart.[30] Therefore, in agreement with expectations, the inter-species divergence of the p40 genes is much less than the divergence from a proposed common ancestral protein which gave rise to both the gastrointestinal peptides and IL-12.

Analysis of the intron/exon junctions of IL-12 and the members of the cytokine receptor family provided an additional piece of evidence supporting an evolutionary relationship among these proteins. In Figure 5, the position of the junctions has been indicated with an arrow above the sequences. There is generally a very close correspondence between the locations of the intron/exon junctions when comparing secretin and glucagon with the IL-4R, the IL2R β chain and human GHR. Using the arrow located at the midpoint of these sequences as a standard, the other junctions are situated on average +/−5 residues away. Although, there is one additional exon included in this region of secretin, it is readily apparent that both the lengths and arrangements of the gene segments from these domains of the proteins are very similar.

DISCUSSION

In order to better understand the diverse biological activities of IL-12 a three dimensional (3-D) model of both subunits has been created through computer modeling. The homology with the human GHR (at least for D2 and D3 of p40) and between p35 and human GH were obvious starting points for assigning coordinates; the similarities between the first domain of p40 and the first domain of CD4 stemmed from the observations reported here. With this 3-D model of IL-12, it is now possible to visualize the spatial orientations of the important regions (e.g., the VTCG adhesion sequence) which have been identified by amino acid sequence homology. The model confirms that these regions are exposed at the protein surface and generally supports the preliminary structural data based on antibody studies.[25] In the predicted structure of p40, the epitope encompassing residues 265–280 is not located near the other defined epitopes as previously suggested by Ling et al.[25] However, it is conceivable that mutations in this region effect the folding of D2 or destabilize pairing with the p35 chain to diminish cytokine activity. Of course, the relative disposition of domains two and three in native p40 may be different from that presented here.

The immunoglobulin-like domains of p40 appear to be very similar to those domains found in the cytokine receptor family, as well as in fibronectin and other adhesion molecules like CD4 and CD2.[44] Recently, the high resolution crystal structure of the interferon-γ receptor (IFN-γRα) has been solved.[45] The analysis reveals that this protein is structurally very similar to the human GHR that has been used here for model building. In comparison with the GHR and IL-12 p40, however, there has been a 40° rotation of the first domain of IFN-γRα relative to the corresponding domain of GHR. In addition, the first domain of IFN-γRα is more upright (with respect to the stalk—domain two) than that of GHR with the angle between the domains increased about 60° in IFN-γRα.

Part of our interest in IL-12 stems from the observation of discrete areas of sequence homology between p40 and adhesion molecules as well as gut-related hormones. Short stretches of homology, such as RGD, VTCG, or VFTD, may occur randomly in a number of unrelated proteins (e.g., the RGD sequence was found 70 times in a database of 1169 proteins).[46] However, several facts argue in favor of a more meaningful interpretation of the findings. First, the RGD and VTCG sequences, which are both involved in cellular adhesion, are found in very close proximity on the same face of D2 in the model of p40. Second, the cytokine receptor family, that includes p40, is homologous to the type III domain of fibronectin[37] which has a functional RGD sequence. Finally, the short segments of homology between IL-12 and VIP/glucagon pointed to larger areas of sequence similarity which included the location of intron/exon junctions in these proteins.

The 3-D structure presented here for the p40 subunit of IL-12 does not imply, however, that the regions which are similar to the gastrointestinal peptides have the same conformation as VIP or glucagon. Rather, we believe that the sequence homology reflects a common evolutionary origin for these proteins or domains. The p40 subunit most likely assumes an alternating β-sheet conformation similar to the GHR[22] and as predicted by Bazan,[47] whereas VIP, glucagon and secretin are believed to have stretches of α-helical conformation and may therefore resemble a module of growth hormone or the other α-helix bundle cytokines.

What are the biological implications of these observations? First, the sequence similarities presented here suggest a common evolutionary ancestry for the gut hormones and the C-terminal domain of the cytokine receptors. More than 20 years ago, Adelson proposed that secreted vertebrate proteins had a common evolutionary origin from digestive enzymes of early organisms and arose by duplication of

genes from the primitive digestive tract.[48] Our analysis would appear to confirm this hypothesis in the case of IL-12 and would extend the concept to cover membrane receptors for cytokines which incidentally may also be found as secreted forms.[16] As shown here, even the locations of the intron/exon junctions of secretin and glucagon resemble those found in the cytokine receptors studied so far. The fact that certain gastrointestinal peptides are similar to lymphocyte growth factor receptors implies that the initial development of the immune system was under the aegis of gut-derived hormones. This early cooperative arrangement is reflected today in the ontogeny of the immune system when lymphoid stem cells initially develop in the yolk sac and fetal liver, both of which are derived from mesodermal tissue.

The second area which may be influenced by these observations is the conceptual framework that integrates the diverse functions of IL-12. We propose that IL-12 plays a broad role in regulating the defenses at mucosal surfaces. This role entails several distinct activities that are mediated by separate regions of the IL-12 heterodimer. 1) In association with the p35 subunit, the p40 partner can mobilize cellular responses at mucosal surfaces. 2) The putative adhesion sequences defined in Figure 4 may allow IL-12: (a) to establish local gradients of cytokine by utilizing the adhesion sequences for extracellular attachment and display, and (b) to compete with parasite adhesion proteins for binding to members of the integrin family, thus preventing entry into host cells. It is interesting to note that both a consensus heparin binding site (residues 4-8: LKKDV[11]) and the VTCG adhesion site (which in some proteins appears to bind sulfated glycoconjugates[49]) are in close proximity in our model of p40. Additional significance for the adhesion sequence in p40 is suggested by the fact, that cellular levels of thrombospondin determine the metastatic potential of certain tumors[50] and furthermore, metastasis may be blocked by the addition of adhesion peptides.[51,52] Thus, the anti-tumor effects of IL-12[9] may derive, in part, from competitive blockade of metastatic pathways. 3) The region of IL-12 that is homologous to the gastrointestinal peptides depicted in Figures 4 & 5 may directly participate in regulation of gut functions by binding (albeit weakly) to hormone receptors thereby controlling secretion, influencing motility or modulating barrier function at mucosal surfaces. A bi-directional regulatory pathway linking the gut with the immune system would appear to exist because, for instance, VIP can also enhance NK cell activity[53] and IgM production in B cells from Peyer's patches.[54]

In summary, it is proposed that IL-12 is a mosaic molecule composed of discrete functional segments which mimic the active sites found in certain other, generally unrelated, proteins. This functional mosaicism would permit cross-regulation of adaptive responses aimed at eliminating pathogens from the gut. Further, it is suggested that certain cytokines/cytokine receptors arose from enterosecretory proteins under selective adaptation of a front line defense of mucosal surfaces. This conceptualization of p40 as a mosaic protein, together with the structural model presented here may suggest additional points to consider when developing therapeutic approaches based on IL-12. A preliminary account of these observations has already appeared as an abstract.[55]

ACKNOWLEDGMENTS

The author thanks Dr. Victor Van Cleave of the Genetics Institute (Andover, MA) for kindly providing information concerning the disulfide pairing in IL-12. This work was supported by funding from the Psychiatry Department at the LSU Medical Center-Shreveport.

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