Minireview

The Interleukin-4-related Lymphokines and Their Binding to Hematopoietin Receptors

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Much of the regulatory activity of T lymphocytes is mediated by a potent set of small proteins, often referred to as lymphokines (1). Lymphokines act on virtually all cells of the hematopoietic system to regulate their growth and differentiation and on many non-hematopoietic cells as well. Table I lists the molecules that are usually included within this group and also presents a set of related factors, often designated as cytokines, that are produced by a variety of cell types.

We have recently pointed out that, among the lymphokines, interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-3 (IL-3) appear to be related (2). We have designated them the interleukin-4 family (Table I). In this communication, we discuss the functional, genetic, and structural characteristics of the family members and of their receptors, with special reference to the requirement for binding and signaling.

Functions of IL-4 Family Members

Table II summarizes the major functions of the members of the IL-4 family. IL-4 itself was first recognized as a co-stimulant of B cell growth. It is also known to be a major regulator of the process through which B cells switch their expression of immunoglobulin class. In the presence of a variety of B cell activators, it causes B cells to switch to the expression and secretion of IgG1 and IgE (3). IL-4 also acts on T cells and on mast cells as a growth factor, regulates macrophage activation, and induces the expression of VCAM-1 on endothelial cells (4).

IL-5 is best known for its ability to regulate eosinophil differentiation (5). It is also a powerful stimulus of B cell growth and immunoglobulin secretion (6, 7), mediating a portion of its activity by inducing the expression of the beta chain of the IL-2 receptor on activated B cells (8).

GM-CSF is known for its function in driving the growth and differentiation of granulocytes, macrophages, and eosinophils. Furthermore, it is also capable of stimulation of multipotential precursors (9). IL-3 is a potent multilineage stimulant. It acts on very immature hematopoietic precursor cells to drive differentiation down a wide range of hematopoietic pathways. In addition, IL-3 supports terminal differentiation of myeloid lineage cells and has particular activity as a stimulant of mast cell growth (10).

A key feature of the members of the IL-4 family is the wide range of actions that they mediate and the variety of cells upon which they act. Furthermore, among these molecules in particular and among the broader set of lymphokines and cytokines, substantial overlap in functions is observed. These properties of pleiotropy and redundancy (1) have made it difficult to predict the major biologic roles of any particular lymphokine. Efforts to determine the unique functions of specific lymphokines are being made through the development of mice in which these genes have been inactivated by homologous recombination. Thus far, results have been reported for IL-2 and IL-4 "knockouts" (11, 12), but work on other lymphokines and cytokines is well under way.

Expression of Members of the IL-4 Family

The members of the family are often co-expressed. Thus, among cloned lines of CD4+ T cells, two major subsets (T1H and T11) have been identified (13). One, T11 cells, produces all four lymphokines (i.e. IL-4, IL-5, GM-CSF, and IL-3) in response to stimulation but fails to produce other lymphokines, such as IL-2 or interferon gamma. In addition, mast cells (14, 15), which bear high affinity receptors for Fc portions of IgE (FccRI), have been shown to produce each of the members of the IL-4 family upon cross-linkage of FccRI but fail to produce IL-2 or interferon gamma.

Linkage and Structure of Genes of IL-4 Family Members

The genes for IL-4, IL-5, GM-CSF, and IL-3 have been mapped to chromosome 5q23-31 and to the syntenic region on mouse chromosome 11 (Fig. 1a) (Ref. 2 and references therein). Physical mapping using pulsed field electrophoresis has shown that the IL-4 and IL-5 genes are from 110 to 190 kb apart in the mouse (16) and 90 to 240 kb apart in the human (17). The IL-3 and GM-CSF genes have been demonstrated to be very close to one another (14 kb apart in the mouse (18); 10 kb apart in the human (19)). The IL-4/IL-5 pair and the GM-CSF/IL-3 pair have been shown to be within 1 cm of one another by RFLP analysis of recombinant inbred mice (20). Physical mapping in the mouse indicates that the genes are more than 600 kb apart (16). In the human, there is suggestive evidence that they may be ~500 kb apart (17).

The genes of the family members are not only closely linked, they also show considerable organizational similarity (Fig. 1b). The GM-CSF, IL-4, and IL-5 genes each consist of 4 exons; the homologous exons for each gene have a very similar lengths and, as described below, encode similar structural elements. The IL-3 gene is also similar to the genes for the other members, with the exception of an apparently "split" third exon (Ref. 2 and references therein).

Structural Features of IL-4 Family Members

The three-dimensional structures of human IL-4 and GM-CSF have been solved, the former by multidimensional heteronuclear magnetic resonance spectroscopy (21, 22) and the latter by x-ray crystallography (23). Both are left-handed four-alpha helix bundles

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Table I

| Cytokines using hematopoietin superfamily receptors |
|---------------------------------------------------|
| The interleukin-4 family                          |
| IL-4                                              |
| IL-5                                              |
| GM-CSF                                            |
| IL-3                                              |

| Interleukin-4-related cytokines                   |
|---------------------------------------------------|
| IL-6                                              |
| Granulocyte colony-stimulating factor             |
| Oncostatin M                                      |
| Leukemia inhibitory factor                        |
| Ciliary neurotrophic factor                       |

| Other cytokines                                   |
|---------------------------------------------------|
| EPO                                               |
| IL-2                                              |
| IL-7                                              |
| IL-12                                             |
| GH                                                |
| Prolactin                                         |

| Cytokines using non-hematopoietin receptors       |
|---------------------------------------------------|
| Interferons                                       |
| Interferon-alpha                                  |
| Interferon-beta                                   |
| Interferon-gamma                                  |

| Cytokines using immunoglobulin-like receptors     |
|---------------------------------------------------|
| IL-1                                              |
| Macrophage colony-stimulating factor              |
| Platelet-derived growth factor                    |
| Stem cell factor, steel factor, or c-kit ligated   |

| Cytokines using nerve growth factor-related receptors |
|------------------------------------------------------|
| Tumor necrosis factor-alpha                          |
| Lymphotoxin                                         |

| Cytokines with uncharacterized receptors            |
|-----------------------------------------------------|
| IL-9                                               |
| IL-10                                              |
| IL-11                                              |

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TABLE II  
Biological properties for the IL-4 family members

| IL-3 | Growth and differentiation of immature pluripotent hematopoietic precursor cells |
| GM-CSF | Growth of mast cells |
| IL-4 | Growth and differentiation macrophages, granulocytes, and eosinophils |
| IL-5 | Stimulation of multilineage hematopoietic precursor cells |

IL-4
- Differentiation of B cells, T cells
- Growth of B cells, T cells, mast cells, thymocytes
- Inhibition of inflammatory responses

IL-5
- Differentiation of B cells, eosinophils
- Growth of B cells, eosinophils

a. human chromosome 5

| GM-CSF | IL-3 |
| IL-5 | |

b. mouse chromosome 11

| GM-CSF | IL-4 |
| IL-5 | |

150 kb

Fig. 1. Organization of the genes encoding IL-4 family proteins. a, genomic organization of the human chromosomal region 11q23-25 containing the four related genes, compared with its murine counterpart on chromosome 11. The maps are deduced from pulse-field electrophoresis (16, 17). Arrows indicate the sense of transcription in the mouse, when known (18) (J.-L. Boulay and W. E. Paul, unpublished data). b, intron-exon structures of the four related murine genes. Solid boxes, open reading frames; open boxes, untranslated regions (see Ref. 2 for references). The size of the introns is not to scale. 6p, base pairs.

Fig. 2. Structure of the IL-4 family proteins. a, secondary structures of the human related proteins, established by X-ray diffraction for GM-CSF (22), NMR analysis for IL-4 (21, 22), and deduced from the use of the heptad algorithm, for IL-3 and IL-5 (28). Wide boxes, α helices named A-D; medium boxes, β-sheets; thin boxes, other structures. b, proposed common spatial structure diagram for human IL-4 and GM-CSF (21-23). Wide boxes, α helices named A-D; medium boxes, β-sheets; lines, other structures. Arrows indicate N-terminal to C-terminal orientation.

Indeed, within these regions, those residues that are the most conserved are hydrophobic amino acids that appear to be involved in interactions between helices forming the internal core of the molecules (22).

IL-4 Family Members Bind to Hematopoietin Receptors

The members of the IL-4 family and several other related molecules bind with high affinity (Kd of 10−8−10−10 M−1) to receptors on target cells. In general, the number of receptors expressed per cell is relatively low, usually less than 1000/cell.

In most instances, the polypeptides that comprise the receptor are members of a related set of membrane proteins, the hematopoietin receptor family (30-34). These molecules possess an ~200-amino acid domain that has two major features, a set of four cysteines in the N-terminal portion of the domain and a conserved Trp-Ser-X-Trp-Ser motif in its C-terminal (membrane proximal) portion (35-37). This domain appears to contain the hematopoietin binding region (35, 36, 38) For this reason, we will refer to this domain as the HB (hematopoietin binding) domain. The cystolic domains of the receptors do not display any homology to one another. Furthermore, they do not display any obvious kinase domains or nucleotide binding sites.

The human IL-3, IL-5, and GM-CSF receptors are heterodimers (Fig. 3a) consisting of an unique α chain with low affinity for the individual ligand and a common β chain that has no detectable intrinsic affinity for the lymphokine. However, the α/β dimer binds lymphokine with high affinity (40, 41). The α chains have a common structural organization, consisting of an N-terminal set of ~190 amino acids followed by single HB domain; the cytosolic domains are ~50 amino acids in length. The β chain consists of two HB domains and has a long cystolic domain, >400 amino acids in length.

The β chain is often present in limiting amounts so that the binding of one lymphokine may diminish the subsequent binding of another member of this group by depleting the pool of common β chains (42, 43). This explains the paradoxical finding of binding competition between molecules that display no detectable cross-specificity for one another's receptors. This model of the receptor consisting of a ligand binding α chain and of a second chain that acts to increase affinity is reminiscent of the strategy employed by the receptors for IL-6 (44) and its congeners (45, 46) and to some extent by receptors for IL-2 (see below for discussion).

In the mouse, the situation appears to be somewhat more complex. A common β chain has been identified that is structur-
Receptors for the IL-4 related proteins. a, identified receptor chains of the IL-4 family. Human IL-3, GM-CSF, and IL-5 receptors are homodimeric. They consist of a specific low affinity a-chain (a, a, and a, different shades of gray) and a common b-chain (white chain) (40, 41). Only a single chain has been identified for the IL-4 receptor, which is not a homologue of either the a or b chains of the receptors of the other family members. Mouse receptors have similar organizations (34, 47-49), except the IL-3 receptor, as discussed in text and in Ref. 43. Conserved amino acids characteristic of the hematopoietin receptor domains are indicated in one-letter code; ec, extracellular domain; mb, membrane; cp, cytoplasmic compartment. b, proposed receptor organization. As outlined in the text, we propose that signaling through the receptors for the interleukin-4 family members and through other hematopoietin receptors requires dimerization of ligand binding domains and long cytoplasmic tails. For the IL-4 receptor, we propose that the single binding chain that has been identified combines the binding and signaling functions which are segregated in the a and b chains of the IL-3, GM-CSF, and IL-5 receptors. We suggest be designated as an a/b chain. Dimerization to form (a/b)2 leads to signal transduction. For the other family members, a dimer of the a/b unit (itself a heterodimer), (a/b)a, is the proposed signaling entity.

Minireview: The IL-4 Family

Dimerization as a Common Mechanism for Signal Transduction through Hematopoietin Receptors

Since IL-4 is structurally homologous to GH as well as to GM-CSF, it is useful to compare its receptor with that for GH (52). The GH binding protein is also a member of the hematopoietin receptor family (see Table I). It is comprised of a single HB domain and has a long cytosolic domain. A single molecule of GH binding protein binds GH with high affinity. Nonetheless, each GH molecule can bind to two molecules of binding protein. Structural analysis of complexes of GH and GH binding protein indicates that GH has two distinct sites, one formed largely by its A and C helices and the other largely by its B and D helices, each of which binds to one molecule of binding protein. To transduce a physiologic signal, GH must bind to two molecules of cell-associated binding protein (52, 53). This strongly suggests that dimerization of the growth hormone binding protein is essential for signal transduction.

The conclusion that dimerization causes signal transduction by hematopoietin receptor family members has been reached from the study of the growth hormone-stimulatory properties of mutants of the EPO receptor. The EPO receptor is also a member of the hematopoietin receptor family (36) and consists of an HB domain and a long cytosolic domain. A mutant in the extracellular domain has been identified that leads to EPO-independent growth in BA/F3 cells (64). These transfected cells are normally dependent upon EPO for growth. This mutant involves the substitution of Cys for Arg at position 129 and presumably leads to dimer formation.

There are strong precedents for dimerization as a critical step in signal transduction. In particular, signaling through membrane tyrosine kinase receptors, such as the platelet-derived growth factor receptor, requires engagement of both sites on a dimer and consequent transphosphorylation of the cytosolic domains of the receptor (55).

Based on the apparent requirement for dimerization in both the GH and EPO systems, we would propose that the single chain IL-4 receptor also transduces signals by the formation of homodimers.

Let us now return to a consideration of the requirements for signal transduction for the other members of the IL-4 family. One possibility is that the formation of a heterodimer of a and b chains as a result of ligand binding is the cross-linking event that initiates the intracellular biochemical events leading to the cellular response to the lymphokine. This is certainly possible, but the fact that the cytosolic domains of the a chains are quite short while the cytosolic domains of the b chains, like those of the IL-4 receptor, the GH binding protein, and the EPO receptor, are long suggests an alternative interpretation. In this model (Fig. 3b), it is the complex of a and b chains that dimerizes, with two molecules of a chain binding to one molecule of ligand and the associated b chains being brought together to generate a signal. In a sense, one can think of the a and the b chains of the IL-3, IL-5, and GM-CSF receptors as having split the functions of the single IL-4 receptor chain into two distinct chains.

Such a model might also be applicable to signal transduction through the IL-6 receptor in which the ligand binding chain must pair with a second chain, gp130, to transduce a signal. The IL-6 binding protein can function even as a soluble molecule, that is a complex of IL-6 and soluble IL-6 receptor will cause cells expressing gp130 to transduce IL-6-mediated signals (38). We postulate that a critical step is dimerization of the gp130 molecule. We suggest that such dimerization of gp130 is also achieved by the binding of leukemia inhibitory factor (46), oncostatin M (45), and ciliary neurotrophic factor (46) to cell surface receptors consisting of both gp130 and a second distinct chain. In each of these instances, high affinity binding depends on the presence of both gp130 and the ligand-specific chain.

We propose that the paradigm of dimerization of hematopoietin receptor family members also holds for signaling through the IL-2 receptor. The IL-2 receptor had been regarded as consisting primarily of two chains (56). One, the b chain, is a hematopoietin receptor family member. It appeared to bind IL-2 with intermediate affinity (10^{-9} M^{-1}). A second chain, the a chain, a non-hematopoietin family member, binds IL-2 with lower affinity (10^{-6} M^{-1}). Cells that express both a and b chains were reported to bind IL-2 with high affinity (10^{-4} M^{-1}) and to respond to low concentrations of IL-2. Cells that express a chains without b chains bind IL-2 with low affinity (but not to IL-2). Cells that express b chains without a chains bind IL-2 with intermediate affinity and display biologic responses, although usually requiring high concentrations of IL-2. Superficially, this model had several similar features to the one discussed here,
except that one of the partner chains lacked an HB domain.

A third chain, γ, has now been identified (57). This chain, which is a hematopoietin family member with a relatively short cystosolic domain (~80 amino acids), has been reported by Takaihata et al. (57) to be essential for internalization and presumably signaling by IL-2 in cells that are α-, β+, or α+, β+. Furthermore, cells expressing a missing chain or one that binds IL-2, but cells that express both β and γ bind IL-2 with intermediate affinity. By contrast, cells that express α chains alone do not bind IL-2 with low affinity.

We interpret these results to indicate that it is the γ/β pair that must dimerize to convey a signal and that these two hematopoietin receptor complexes are the formal analogues of the α/β chains of IL-3, IL-5, and GM-CSF receptors and the IL-6R/gp130 chains of the IL-6 receptors.

The concept that it is the dimerization of two hematopoietin receptor chains with long cystosolic domains is required for signal transduction raises a very interesting question about the specific properties of activity of IL-5, IL-6, and GM-CSF. In the human, a single β chain is used by each of these receptors and in the mouse IL-5 and GM-CSF use a common β chain. If, as we propose, it is dimerization of the β chain that is critical to signal transduction, one would anticipate that cells that bear ligand-specific α chains for two or three of these factors would display similar responses to each ligand. Indeed, it has been shown that the pattern of expression of the ligand-specific α chain. However, both cell-specific expression of α chains and differences in expression after activation or other types of treatment could offer greater flexibility in the regulation of responsiveness to these molecules.

Conclusion

When first described, the lymphocytes appeared to be a heterogeneous collection of molecules related to one another only by the fact that they were produced by cells of the immune system in response to antigenic stimulation and that they regulated key aspects of immune and inflammatory responses as well as having a major function in hematopoiesis. The recognition that their receptors are members of a common family and that a subset of these molecules is closely related indicates that their study as a group, indeed, is valid and that it is likely that general rules governing their production and function will continue to emerge. In this review, we have discussed the genetic linkage and the common features of the genetic organization and structure of the members of the interleukin-4 receptor family, placing special emphasis on their receptors. We have proposed that dimerization of receptors is essential for signal generation and have discussed this in the context of the wider set of molecules of which this family is a part. Nonetheless, it is clear that the recognition of this close family structure should lead to other insights, particularly of a functional nature about the regulation of their production and of their physiologic control of immune and inflammatory responses.

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