

Molecular mechanisms in allergy and clinical immunology

Series editors: William T. Shearer, MD, PhD, Lanny J. Rosenwasser, MD, and Bruce S. Bochner, MD

Biology of common β receptor–signaling cytokines: IL-3, IL-5, and GM-CSF

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IL-3, IL-5, and GM-CSF are related hematopoietic cytokines that are important for allergic inflammation. The receptors for human IL-5, IL-3, and GM-CSF are members of the hematopoietin receptor superfamily and are comprised of a cytokine-specific α chain and the common β chain that is shared among these cytokines for signaling. Each of these cytokines contributes to the differentiation and function of leukocyte subpopulations and have clinical importance in protective immunity and in the pathophysiology of a spectrum of immunologic diseases that are as diverse as allergy and asthma, pulmonary alveolar proteinosis, neurodegenerative diseases, and malignancies. Delineating the biology of these cytokines is enabling the development of new strategies for diagnosing and treating these diseases and modulating immune responses. (*J Allergy Clin Immunol* 2003;112:653-65.)

Key words: IL-3, IL-5, GM-CSF, β c, allergic inflammation, asthma, hematopoiesis, cytokine physiology

Cytokines are a pleiotropic group of molecules that are usually secreted by cells of the hematopoietic lineage and provide autocrine or paracrine regulation of hematopoietic cell differentiation or regulation of immune cell function.¹⁻³ Structural features among cytokines allow for their classification into distinct subsets of cytokine families. Likewise, structural features distinguish subsets of cytokine receptors. IL-3, IL-5, and GM-CSF are members of the short-chain 4- α -helical bundle subset of hematopoietic cytokines and are further distinguished by their shared use of the common β receptor (β c) for signaling.²⁻⁴ However, β c is only a subunit of

Abbreviations used

β c:	Common β receptor
ERK:	Extracellular signal–regulated kinases
GM-CSFR α :	Granulocyte-macrophage colony-stimulating factor receptor α
IL-3R α :	IL-3 receptor α
IL-5R α :	IL-5 receptor α
β _{IP} :	β c Intracytoplasmic proteolysis
JAK:	Janus kinase
JNK:	c-Jun amino-terminal kinase
MAPK:	Mitogen-activated protein kinase
PAP:	Pulmonary alveolar proteinosis
PI3-K:	Phosphatidylinositol 3-kinase
PIAS:	Protein inhibitors of activated STAT
PIP ₃ :	Phosphatidylinositol 3,4,5-triphosphate
SH2:	Src homology 2
SHP:	Src homology protein tyrosine phosphatase
SOCS:	Suppressors of cytokine signaling
STAT:	Signal transducer and activator of transcription

the complete heterodimeric receptor for these cytokines and alone is not able to bind any of the cytokines.⁵⁻⁷ IL-3, IL-5, and GM-CSF each has a unique α receptor subunit (IL-3R α , IL-5R α , or GM-CSFR α , respectively) that only binds its specific ligand.²⁻⁷ On cytokine binding to its specific R α , β c is recruited to engage the cytokine, thereby providing the necessary physical and conformational proximity of the R α and β c subunits to initiate intracellular signaling and a physiologic effect (Fig 1).

Collectively, IL-3, IL-5, and GM-CSF synergize for the differentiation and function of myeloid cells and are of particular importance in allergic inflammation.¹⁻⁸ In addition, these cytokines are important for protective immunity and in the pathophysiology of diseases as diverse as pulmonary alveolar proteinosis (PAP), neurodegenerative disorders, and some malignancies.⁹⁻¹² Recent studies also demonstrate a role for these cytokines in modulating T_H1 and T_H2 immune responses.¹³⁻¹⁶ Thus unraveling the biology of IL-3, IL-5, and GM-CSF is helping to elucidate the process of hematopoiesis, mechanisms of protective and pathologic immune responses, and molecular targets for modulation of these cytokine-mediated responses.

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Supported in part by grants from the National Institutes of Health (AI 36936 and AI50686) and the Cullen, Fondren, Mendenhall, and Trammell Foundations.

Received for publication May 16, 2003; revised August 12, 2003; accepted for publication August 13, 2003.

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0091-6749/2003 \$30.00 + 0
doi:10.1067/mai.2003.1796

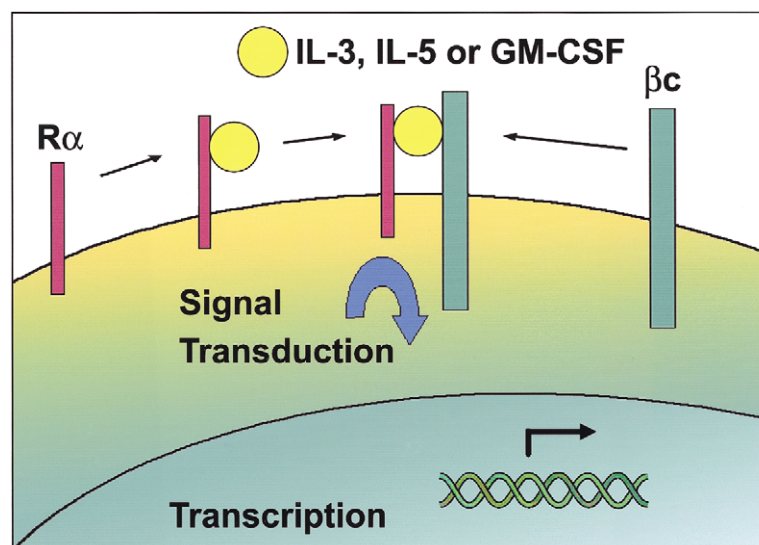


FIG 1. Assembly of the IL-3, IL-5, and GM-CSF receptors. IL-3R α , IL-5R α , and GM-CSFR α exist as monomers on unstimulated cells. Each R α chain provides cytokine binding specificity. On ligand binding, β c is recruited to the R α /ligand complex and interacts with the R α -bound cytokine to activate signal transduction and a physiologic response.

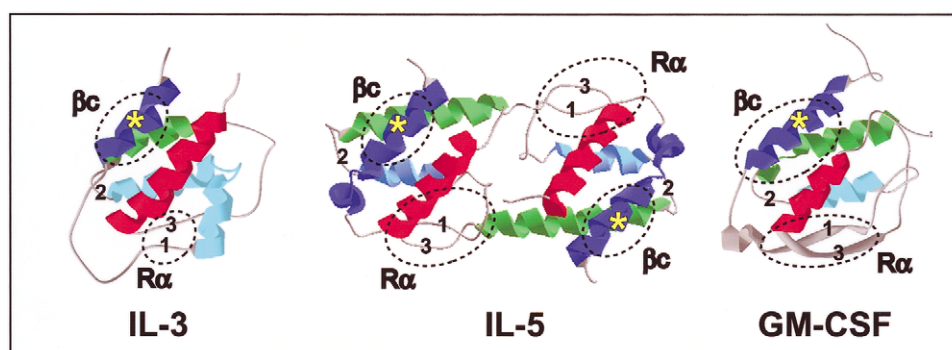


FIG 2. Structure of IL-3, IL-5, and GM-CSF. The ribbon structures of IL-3, IL-5, and GM-CSF illustrate the 4- α -helical bundle motifs. IL-3 and GM-CSF exist as monomers. IL-5 exists as an interdigitating homodimer that imparts a pair of 4- α -helical bundle motifs. The domains for cytokine-specific R α binding and for β c engagement are depicted by the *broken circles*. Data are derived from the protein data bank and represent structures derived from nuclear magnetic resonance or x-ray diffraction. Loops connecting each helix are designated 1 to 3. The glutamate residue in each A helix crucial for β c engagement is depicted by the *yellow asterisk*. *Blue*, Amino-terminal A helix; *green*, B helix; *aqua*, C helix; *red*, carboxy-terminal D helix.

FUNCTIONAL STRUCTURE OF IL-3, IL-5, AND GM-CSF

The functional structure of hematopoietic cytokines is characterized by orientation of their 4 α helices in an up-up-down-down antiparallel conformation that is enabled by loop sequences between each of the helices.^{2,4} IL-3 and GM-CSF can assume this functional conformation by means of the intramolecular folding of a single polypeptide chain (Fig 2, left and right).⁴ However, IL-5 is unique in that 2 identical polypeptide chains interdigitate to achieve a functional conformation (Fig 2, middle), which is characterized by a pair of 4- α -helical bundles formed by the sharing of the fourth helix of one chain of

IL-5 with the first 3 helices of the other chain of IL-5 (ie, a homodimer in which there are 2 functional motifs for binding to IL-5R α and 2 functional motifs for binding to β c).¹⁷⁻¹⁹ This unique conformational requirement for IL-5 is the consequence of a structurally short loop 3 that physically impedes the folding of a single chain of IL-5 into the required 4- α -helical bundle motif.^{18,19} The domains within IL-3, IL-5, and GM-CSF that engage their cognate R α and the β c have also been elucidated (Fig 2, dashed lines).²⁰⁻²³ The R α binding domain for each cytokine includes their antiparallel first and third loops, whereas the β c binding domain is centered around a glutamate residue (residue 23 in IL-3, residue 13 in IL-5, and residue 21 in GM-CSF) within the first α -helical

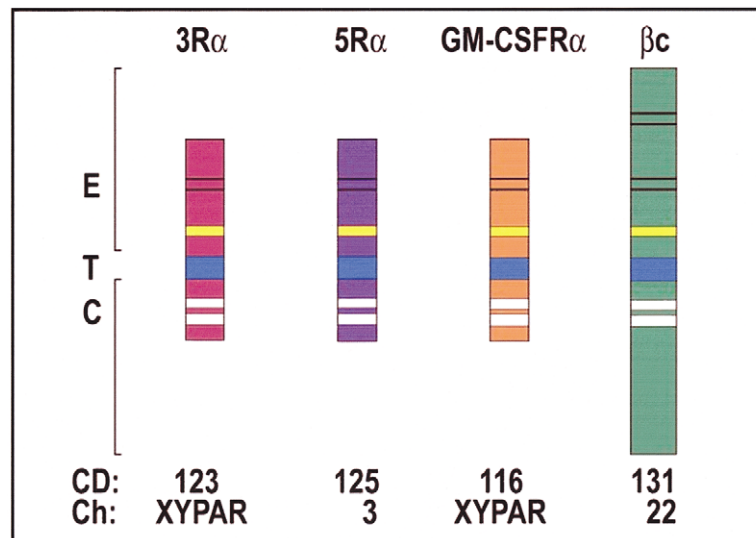


FIG 3. Structural features of the IL-3Rα, IL-5Rα, GM-CSFRα, and βc. The extracellular domains (E) of each Rα contains a membrane proximal WSXWS motif (yellow) and a homology module containing 2 fibronectin type III domains with paired cysteine residues (solid black lines). The βc E domain is similar but larger, with 2 homology modules and 2 pairs of cysteine residues. The transmembrane domain of each receptor is blue. Whereas IL-3Rα, IL-5Rα, and GM-CSFRα have short cytoplasmic domains (C), βc has a much longer C domain for signaling. The white boxes represent Box1 and Box2 JAK binding sites. The IL-5Rα and the GM-CSFRα also exist as soluble external domains. The CD designation and chromosome (Ch) localization for each receptor subunit is indicated. XY PAR, X and Y chromosome pseudoautosomal region.

bundle (Fig 2, yellow asterisks). In addition to sharing the βc receptor subunit for signaling, IL-3, IL-5, and GM-CSF also have the common feature of being proximately encoded on human chromosome 5, suggesting a common ancestral relationship.²⁴ Further delineation of the receptor binding domains for these cytokines has the potential to enable the rational design of antagonist molecules for therapeutic application.

STRUCTURE OF THE IL-3, IL-5, AND GM-CSF RECEPTORS

The hematopoietin receptor family is characterized by a 200-amino-acid extracellular homology module containing 2 fibronectin type III domains with conserved sequence features.¹⁻³ These conserved features include a membrane proximal WSXWS motif and membrane distal paired cysteine residues (Fig 3). The IL-3Rα, IL-5Rα, and GM-CSFRα each exhibit these features. The βc external domain is distinguished from each of these Rα external domains by being longer because of 2 pairs of the fibronectin domains (Fig 3). The IL-3Rα, IL-5Rα, and GM-CSFRα each exist as a transmembrane molecule, the cytoplasmic domain of which contains conserved motifs necessary for initiation of signaling.¹⁻³ The longer βc cytoplasmic domain contains sequence necessary for completion of signal transduction. In addition, IL-5Rα and GM-CSFRα exist as soluble external domains (sIL-5Rα and sGM-CSFRα) that are generated by means of

alternative splicing of the *IL-5Rα* and *GM-CSFRα* genes and are capable of competing with the transmembrane IL-5Rα and GM-CSFRα for binding IL-5 and GM-CSF, respectively.^{25,26} Because these soluble receptor isoforms lack cytoplasmic domains, they are unable to participate in agonistic signaling. Whether sIL-5Rα and sGM-CSFRα are physiologic for downregulation of IL-5 and GM-CSF responses or serve as protective carriers of these cytokines to distant sites is not resolved.

In contrast to a common chromosome region for the cytokine genes,²⁴ their receptor genes are dispersed (Fig 3).^{27,28} The genes encoding IL-3Rα and GM-CSFRα both colocalize to the homologous distal pseudoautosomal regions of the short arms of the X-Y sex chromosomes and hence behave as autosomally inherited genes.²⁸ However, the *IL-5Rα* gene is encoded on chromosome 3, and the gene encoding βc is encoded on chromosome 22.²⁷ Despite the encoding of *IL-5Rα* on a chromosome distinct from that for *IL-3Rα* and *GM-CSFRα*, their general structure is similar, yet sufficiently unique at key residues so as to have cytokine specificity. Cellular responsiveness to these cytokines is dictated by the repertoire of cytokine Rα chains expressed, and receptor activation is associated with disulfide-linked heterodimerization of the Rα with βc.^{29,30} In addition, distinct Rα cytoplasmic domain signaling cascades can confer cytokine-specific cellular activation.³¹⁻³³ Bioinformatic analysis of the IL-3Rα and GM-CSFRα defined a tripeptide sequence adjacent to the proline-rich region of their cytoplasmic domains that

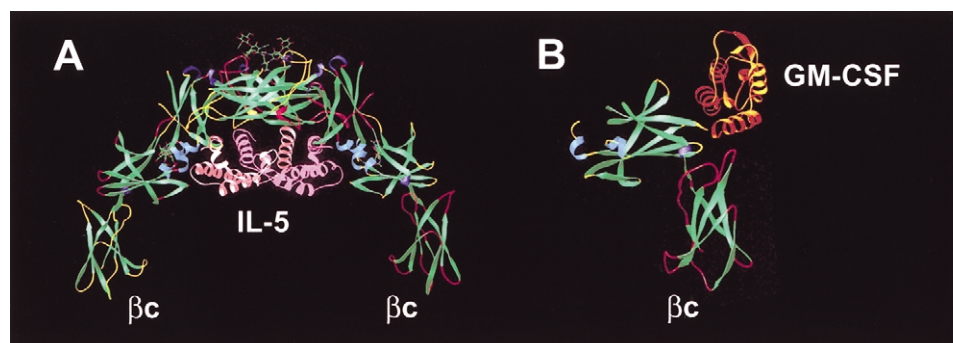


FIG 4. Structure of βc . **A**, The ribbon diagram depicts the x-ray diffraction-derived structure for the external domain of βc . The arch-like conformation is comprised of 2 chains of βc that interdigitate. The potential βc domains for cytokine engagement are illustrated by using IL-5. **B**, An alternative ribbon model for the potential structure for the external domain of a βc monomer. If such a structure exists, the potential orientation of a βc -engaging cytokine is illustrated with GM-CSF. Reproduced and modified with permission from Carr et al.³⁴

affected cytokine-specific function.³³ For IL-5 signaling, syntenin/SOX-4 activation was unique to the IL-5R α cytoplasmic domain.³¹

Recently, a crystal structure of the complete βc extracellular domain was solved, and surprisingly, it is unlike other described cytokine receptors, which exist as monomers.^{34,35} The βc structure revealed an interdigitating homodimer with an arch-like conformation (Fig 4, A). The βc interlocking homodimer structure is also distinct from that of monomeric cytokine receptors that are homodimerized by ligand binding, such as growth hormone receptors and erythropoietin receptors. Because the cytoplasmic domains of the homodimeric βc are too far apart to activate each other for signaling, βc activation would require proximate association of either monomer within βc with a ligand-bound IL-3R α , IL-5R α , or GM-CSFR α .^{34,35}

Although crystallization of the βc external domain yielded a homodimeric structure with exposed residues predicted as binding sites for IL-3, IL-5, or GM-CSF, immunoprecipitation of βc from cell membranes reveals both dimer and monomer forms of βc .³⁶ Therefore it is possible that βc can also exist as a monomer with a predicted structure that is capable of engaging IL-3, IL-5, or GM-CSF (Fig 4, B).

INTRACELLULAR SIGNALING CASCADES

Similar to all cytokines and growth factors, IL-3, IL-5, and GM-CSF exert their biologic responses by first binding their cognate receptors on the surface of target cells.³⁷⁻⁴² Specific ligand binding of an R α chain recruits the βc chain, which leads to heteromeric receptor assembly and culminates in the initiation of signal transduction. It is the activation of intracellular signaling that rapidly reprograms gene expression of the target cell to alter its behavior. To date, at least 3 principal signaling pathways have been described for these cytokines: the

Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, the mitogen-activated protein kinase (MAPK) pathways, and the phosphatidylinositol 3-kinase (PI3-K) pathway (Fig 5).³⁸⁻⁴²

The JAK/STAT pathway

The JAK/STAT pathway is used by all cytokines and some growth factors for transmitting extracellular signals through transmembrane receptors directly to promoters of target genes in the nucleus of activated cells.³⁷ The cellular efficiency of this pathway is demonstrated by the achievement of transcriptional gene regulation in the absence of second messengers. Moreover, the importance of this pathway is underscored by its evolutionary conservation from slime molds to human subjects.³⁷ To date, there are 4 JAKs (JAK1, JAK2, JAK3, and TYK2) and 7 STATs (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6) described in mammalian cells.³⁷⁻⁴²

IL-3, IL-5, and GM-CSF receptor binding leads to activation of the receptor-associated JAK2 and JAK1 kinases by means of transphosphorylation of the 2 kinases after oligomerization of the receptor subunits (Fig 5).³⁸⁻⁴² Although JAK2 is the predominant βc -activating kinase, members of the Src family of kinases, such as Lyn, Fyn, Syk, Hck, and Btk, have also been reported to be activated by these 3 cytokines.⁴³⁻⁴⁵ Lyn and Syk are reported to be essential for the activation of the anti-apoptotic pathway induced by βc activation in freshly isolated human eosinophils.⁴⁵

JAK activation results in tyrosine phosphorylation of βc on 6 critical tyrosine (Y) residues: Y577, Y612, Y695, Y750, Y806, and Y866.³⁸⁻⁴² Of these phosphorylated residues, Y612, Y695, and Y750 serve as docking sites for the Src homology 2 (SH2) domains of 2 members of the STAT family, STAT1 and STAT5. The 2 STATs are recruited to the receptor complex by their SH2 domains, become tyrosine phosphorylated on critical residues in

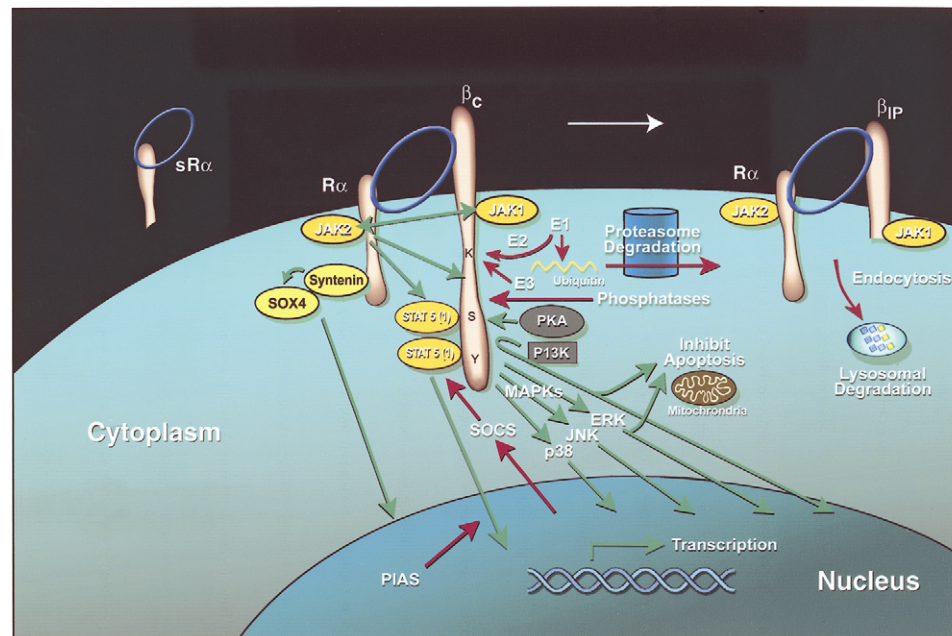


FIG 5. Activation and inactivation pathways of the β_c -engaging cytokines. Cytokine engagement of R_α by IL-3, IL-5, or GM-CSF leads to the recruitment of β_c , which results in the activation of the JAK/STAT pathway; the MAPK cascade pathway, including ERK, JNK, and p38 pathways; and the PI3-K pathway. Activation results in phosphorylation of tyrosines (Y) and a serine (S) in the β_c cytoplasmic domain. Once activated, the cytoplasmic domain of β_c is rapidly ubiquitinated on lysine (K) residues by the ubiquitination machinery consisting of E1 (ubiquitin activator), E2 (ubiquitin conjugator), and E3 (ubiquitin ligase), followed by proteasome degradation to terminate agonistic signaling. Proteasome degradation of the β_c cytoplasmic domain results in the generation of β_{IP}/R_α /cytokine complexes, which are then endocytosed and degraded in lysosomes. In addition, SOCS and PIAS downregulate signaling by inhibiting the JAK and STAT pathways, and phosphatases downregulate signaling by dephosphorylation. IL-5 and GM-CSF have soluble R_α (s R_α) isoforms that can potentially act as antagonists of the transmembrane receptor isoforms (upper left corner). Syntenin and Sox4 are molecules specifically activated by IL-5 through the IL-5 R_α .³¹ Green arrows represent activation pathways, and red arrows indicate inactivation pathways.

their carboxyl termini, and then homodimerize or heterodimerize by means of reciprocal SH2 and phosphotyrosine interactions. Once the STATs dimerize, they translocate to the nucleus (Fig 5) and bind to specific enhancer sequences in the promoters of activated genes.

Mutation of 4 β_c tyrosine residues (Y577, Y612, Y695, and Y750) completely blocked IL-5-induced STAT5-mediated transcription, suggesting that STAT5 docking to the β_c receptor is required for its activation.⁴⁶ Studies using dominant negative mutants of either JAK2 or STAT5 also demonstrated that the JAK/STAT pathway is important for cytokine-induced proliferation and differentiation.^{46,47}

The MAPK pathways

The MAPK pathways include the extracellular signal-regulated kinase (ERK), c-Jun amino-terminal kinase (JNK), and p38 signaling cascades (Fig 5).³⁸⁻⁴² These pathways are vital mediators of a number of cellular responses, including growth, proliferation, and survival.

These pathways are activated by numerous growth factors and cytokines, including IL-3, IL-5, and GM-CSF.³⁸⁻⁴²

Activation of the ERK pathway by these cytokines begins with the phosphorylation of Y577 on β_c by JAK2.³⁸⁻⁴² Once phosphorylated, this β_c residue becomes a recruitment site for the cellular substrate Shc, which then becomes tyrosine phosphorylated, allowing it to interact with the SH2 domain of the growth factor receptor-bound adaptor protein Grb2. The Shc/Grb2 interaction leads to an association with the mammalian son of sevenless homologue, the nucleotide exchange factor for Ras, which, in turn, results in the sequential activation of Ras, Raf-1, MEK, and ERK. The β_c cytoplasmic region responsible for activation of this pathway is between amino acids 544 and 763.⁴⁸ The downstream targets of the ERK pathway are the transcription factors ATF2 and TCF, which eventually activate c-Fos and c-Jun and contribute to cytokine-induced proliferation.⁴⁸ In addition, the ERK pathway is involved in preventing apoptosis by upregulating expression of the genes encod-

ing bcl-2 and bcl-x in response to these cytokines.⁴⁹⁻⁵³ For example, IL-3 stimulation or overexpression of activated Ras (an upstream ERK activator) rapidly increases expression of bcl-2 and bcl-x, thereby preventing apoptosis.^{49,50} Similarly, overexpression of bcl-2 prevents apoptosis induced by IL-3 withdrawal.⁵² Furthermore, expression of a dominant-negative MAPK kinase (another upstream ERK activator) suppressed IL-3-dependent cell survival.⁵³ In addition to activating survival genes, the ERK pathway prevents apoptosis by inhibiting the proapoptotic factor BAD.^{54,55} The ERK pathway stimulates selective phosphorylation of BAD on serine 112, whereas the PI3 kinase/Akt/PKB pathway phosphorylates BAD on serine 136.^{54,55} Consequently, the dual-phosphorylated BAD binds to the adaptor protein 14-3-3, which leads to sequestration of BAD in the cytoplasm and inhibition of its proapoptotic activity. Taken together, these data suggest that the ERK pathway inhibits apoptosis in 2 ways, by inhibiting BAD activity and by upregulating expression of bcl-2 and bcl-x.

The 2 other cascades activated by these cytokines are the JNK cascade and the p38 cascade (Fig 5).⁵⁶⁻⁵⁹ JNK phosphorylates the c-Jun transcription factor, whereas the p38 MAPKs activate the ATF transcription factor. Although not known to participate in controlling cell survival, the JNK and p38 cascades signal in parallel with the ERK cascade for cell growth and effector function.

The PI3-K cascade

The PI3-K pathway regulates multiple cellular processes (Fig 5), such as proliferation, growth, cell size, apoptosis, and cytoskeletal rearrangement.^{60,61} PI3-K is a heterodimeric lipid kinase composed of a regulatory subunit (p85) and a catalytic subunit (p110), each encoded by different genes. On cytokine stimulation, PI3-K phosphorylates phosphatidylinositol lipids at the D-3 position of the inositol ring at cellular membranes to produce the signaling molecule, phosphatidylinositol 3,4,5-triphosphate (PIP₃).^{60,61} As a second messenger, PIP₃ interacts with other signaling proteins through pleckstrin homology domains and coordinates and amplifies the activated signal.

IL-3, IL-5, and GM-CSF rapidly activate PI3-K in a tyrosine phosphorylation-dependent manner, and there is a direct link between IL-3-stimulated β c serine phosphorylation, PI3-K activation, and hematopoietic cell survival.⁶²⁻⁶⁴ IL-3 stimulation results in the activation of protein kinase A, which phosphorylates serine 585 on β c.⁶³ This β c phosphorylation event becomes a recruitment site for the adaptor protein 14-3-3, which in turn recruits the p85 and p110 subunits of PI3-K to the receptor complex.⁶³ At this point, an unidentified kinase phosphorylates PI3-K and leads to the generation of PIP₃, resulting in the recruitment and binding of the serine-threonine kinase Akt/PKB.⁶³ IL-3 stimulation also induces phosphorylation of BAD through the Akt/PKB pathway, resulting in inhibition of apoptosis.⁶⁴ In aggregate, these studies suggest that cytokine signaling through the β c serine 585 is important for hematopoietic

cell survival, possibly through the PI3-K/Akt/BAD anti-apoptotic pathway.

Signal termination

Multicellular organisms have developed many down-regulatory mechanisms to ensure that the execution of specific signals are of adequate strength and are delivered for the appropriate time.⁶⁵ For this reason, activation of signaling pathways by means of extracellular stimuli is transient. Serious consequences can arise when there is a lack of signal termination, as seen with some cancers and some developmental and inflammatory disorders.

To date, several negative feedback mechanisms have been described for the IL-3, IL-5, and GM-CSF receptor system (Fig 5, red arrows).⁶⁶⁻⁷⁵ One mechanism is the activation of cytosolic tyrosine phosphatases that control the level of ligand-induced phosphorylated substrates.^{66,67} The tyrosine phosphatase Src homology protein tyrosine phosphatase 1 (SHP1; also known as HCP, SHPTP1, and SHP) is involved in downregulating β c activation because its overexpression results in suppression of cell growth in response to IL-3.⁶⁶ In contrast, SHP2 appears to be a positive regulator of β c signaling.⁶⁷ Thus phosphatases can participate in both agonistic signaling and the inhibition of signaling.

Another mechanism that antagonizes IL-3, IL-5, and GM-CSF agonistic signals is induction of the suppressors of cytokine signaling (SOCS) family of small SH2-containing proteins, which include SOCS1-7.⁶⁹⁻⁷² (also referred to as JAB,⁶⁹ a JAK-binding protein), SSI⁷⁰ (STAT-induced STAT inhibitors), and CIS1⁷¹ (cytokine-inducible SH2-containing protein). SOCS family members negatively regulate signaling by mechanisms that include inactivation of the JAKs, blocking access of the STATS to receptor binding sites, and ubiquitination of signaling proteins for subsequent targeting by the proteasome.⁷² CIS1 is reported to downregulate STAT5 signaling, whereas SOCS1 negatively regulates JAK2 activity.⁷²

STAT function is also negatively regulated by another family of proteins termed protein inhibitors of activated STAT (PIAS).^{72,73} PIAS1 binds activated STAT1 and blocks its DNA binding.⁷³ To date, a STAT5-specific PIAS has not been identified.

A further mechanism for terminating IL-3, IL-5, and GM-CSF signal transduction is receptor endocytosis and degradation.^{74,75} For β c, specific proteasome inhibitors revealed that after IL-5 ligation of its receptor, β c is downregulated by ubiquitination and proteasome degradation of its cytoplasmic domain, resulting in the generation of cytoplasmically truncated β c products termed β c intracytoplasmic proteolysis (β c_{IP}; Fig 5).⁷⁵ Similar results were observed with all 3 β c-engaging cytokines.⁷⁵ Structurally, β c_{IP} contains the extracellular domain, the transmembrane domain, and a small part of its cytoplasmic domain. However, β c_{IP} is devoid of all tyrosine and serine phosphorylation because proteasomes degrade the large cytoplasmic domain containing all of these residues.⁷⁵ Moreover, inhibition of β c proteasome degra-

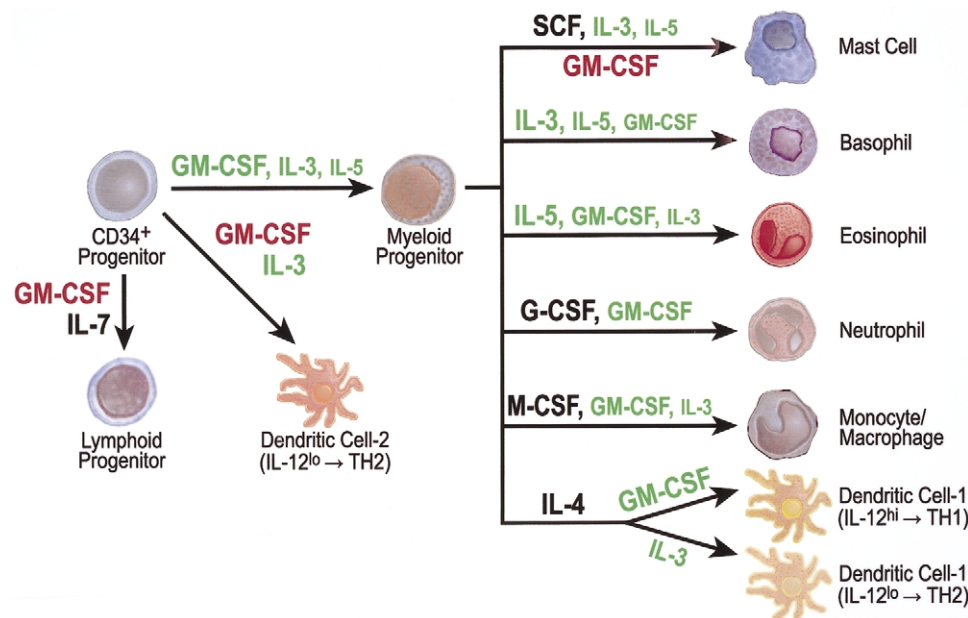


FIG 6. Major effects of IL-3, IL-5, and GM-CSF on leukocyte subsets. Positive stimulation is indicated by *green letters*. Inhibition is indicated by *red letters*. Non-βc-engaging cytokines that are crucial for differentiation are indicated in *black*. The relative biologic effect (reflecting relevant receptor density on the cell surface) is indicated by the size of the cytokine lettering.

TABLE I. Physiology and pathophysiology of IL-3, IL-5, and GM-CSF

Cytokine	Major source	Effect on hematopoiesis	Physiologic and pathophysiologic role	Gene deletion phenotype
IL-3	T cells, macrophages, stromal cells	Growth, differentiation, and survival of progenitor cells	Allergic inflammation; parasite immunity; growth of myeloid, monocytic, and lymphocytic malignancies	Blunted mast cell and basophil response to parasitic infection
IL-5	T _H 2 cells, eosinophils, mast cells	Differentiation, activation, and survival of eosinophils	Allergic inflammation, asthma and airway remodeling, parasite immunity	Reduced numbers of eosinophils ± delay of CD5 ⁺ B-cell development ± risk for parasitic infection
GM-CSF	T cells, macrophages, endothelium, fibroblasts	Growth and differentiation of dendritic cells, myelomonocyte progenitors and granulocytes	Allergic inflammation, growth of myeloid and monocytic malignancies, surfactant metabolism, anti-GM-CSF autoantibodies cause PAP	PAP

dation results in prolonged activation of βc, JAK2, STAT5, and SHP2.⁷⁵ This observation suggested that activation of the ubiquitin proteasome pathway is a very rapid and efficient mechanism for complete termination of βc signal transduction. After proteasome degradation of the βc cytoplasmic tail, the remaining truncated IL-5R complex (5Rα and β_{1P}) is endocytosed and degraded in the lysosomes.⁷⁵ This downregulatory process results in transient heterotypic desensitization to other βc-engaging cytokines, thus preventing the stimulated cell from being further activated by more than one βc-engaging cytokine. This

paradigm of heterotypic desensitization exists for each of the βc-engaging cytokines.⁷⁵

PHYSIOLOGY AND PATHOPHYSIOLOGY

Leukocytes differentiate from a small population of pluripotent progenitor cells under the influence of hematopoietic cytokines that include not only IL-3, IL-5, and GM-CSF but also stem cell factor, granulocyte colony-stimulating factor, monocyte colony-stimulating factor, IL-4, and IL-7 among many others.⁷⁶ Each of these cytokines contributes to the specific growth and

differentiation of progenitor cells to produce fully activated effector cells. Although IL-3, IL-5, and GM-CSF each contributes to the growth, differentiation, and activation of several leukocyte subpopulations, their activities are not totally redundant (Table I and Fig 6).

IL-3

IL-3 plays an important role in the growth and differentiation of CD34⁺ progenitor cells into basophils and mast cells, myeloid-derived dendritic cells and nonmyeloid-derived dendritic cells, and, to a lesser extent, eosinophils and monocytes-macrophages (Fig 6).^{1-3,9,12-16,76,77} This pattern of IL-3 responsiveness corresponds to the relative cellular expression of IL-3R α .⁷⁷ Although IL-3 is not essential for the development of mast cells and basophils under physiologic conditions, it is necessary for supporting increased numbers of tissue mast cells, enhanced basophil production, and protective immunity to parasites, as evidenced in mice with and without deletion of the *IL-3* gene that were infected with *Strongyloides venezuelensis*.⁹ Interestingly, disruption of the *IL-3* gene or the *IL-3R α* gene does not result in alteration of basal hematopoiesis or growth, development, or longevity of the animals.⁹ However, IL-3, but not IL-5 or GM-CSF, prevents basophil apoptosis in vitro through the activation of PI3-K.⁷⁸ IL-3 also promotes stem cell factor–induced mast cell differentiation in a dose-dependent manner and enhances Fc ϵ R1-dependent mediator release.⁷⁹

A potential role for IL-3 in the pathophysiology of inflammation in the central nervous system has been suggested.⁸⁰⁻⁸² Data to support this notion include the observation in rats that microglia can produce and respond to IL-3 and that β c expression in the brain is restricted to the microglial cells, suggesting that IL-3 might function in an autocrine feedback loop.⁸⁰ In vitro IL-3 stimulation of microglial cells induced their proliferation and the formation of multinucleated giant cells.⁸¹ The clinical relevance of these observations is suggested by the detection of IL-3 in postmortem brain tissue from patients with Alzheimer's disease.⁸² Taken together, these data suggest that IL-3 might mediate increased activation of microglial cells and the subsequent development of neurodegeneration.

The expression of IL-3R α on a wide range of hematologic malignancies is of potential importance as a maker of tumor load.^{12,83} Although not normally expressed by B cells, increased expression of IL-3R α is observed in approximately 40% of patients with B cell–acute lymphocytic leukemia or acute myeloid leukemia.⁸³ Furthermore, the increased expression of IL-3R α is associated with enhanced blast proliferation, increased cellularity, and a poor prognosis.⁸³ Whether IL-3R α is a potential target for therapeutic intervention in these malignancies remains to be determined.

Recently, a novel role for IL-3 in modulating type 1 dendritic cell function was reported.¹³ When human type 1 dendritic cells were derived in vitro with IL-3 rather than GM-CSF, the dendritic cells preferentially induced T_H2 responses (Fig 6). In contrast, type 1 dendritic cells

derived with GM-CSF preferentially induced T_H1 responses. This difference was the consequence of decreased IL-12 production by IL-3–derived type 1 dendritic cells. In addition, differentiation of type 2 dendritic cells requires IL-3, and these cells also produce low quantities of IL-12 and promote T_H2 responses (Fig 6). Thus IL-3 might become an important molecule in the development of strategies to modulate T_H1 and T_H2 immune responses.

IL-5

IL-5 is produced predominantly by T_H2 cells, as well as activated mast cells and eosinophils.^{76,84,85} The *IL-5* gene (along with other T_H2 genes) is activated under the control of the transcription factor GATA-3.⁸⁶ The major target cells for IL-5 are eosinophils and their progenitors, which express greater numbers of IL-5R α than basophils and mast cells (Fig 6).^{77,87} IL-5 stimulation of eosinophils leads to their activation, with upregulation of CD11b and inhibition of apoptosis.^{88,89} The earliest IL-5R α –expressing progenitor cells are CD34⁺ cells found in bone marrow, as well as peripheral tissues and blood.⁸⁷ These cells increase in number after allergen challenge and are mobilized into the peripheral blood as differentiated eosinophils.⁸⁷ Although eosinophils express IL-3R α and GM-CSFR α in addition to IL-5R α and all 3 cytokines can affect eosinophil activation and survival, IL-5 is the key cytokine for production of normal numbers of eosinophils. This is evidenced by the few eosinophils present in mice engineered for deletion of either IL-5 or IL-5R α .^{90,91}

Several lines of evidence indicate that IL-5 is important in the pathophysiology of allergic inflammation and asthma.⁹²⁻⁹⁵ Peripheral blood eosinophil counts increase within 24 hours of allergen challenge, and eosinophil infiltration of the lungs is a hallmark of asthma.⁹³ Furthermore, eosinophil-derived mediators are both toxic to the bronchial epithelium and can cause bronchospasm.⁹³ In addition, IL-5 levels, T_H2 T cells, and eosinophils are all increased in the bronchoalveolar lavage fluid of asthmatic patients, and the levels correlate with the severity of disease.⁹⁴ Similar increases of IL-5, T_H2 cells, and eosinophils are found in the bronchial mucosa of patients with severe asthma.⁹⁵

Murine models of asthma also support a role for IL-5. The most compelling data come from studies in which mice deficient for IL-5 do not have airway eosinophilia or bronchial hyperreactivity after airway allergen challenge.⁹⁶ However, when these animals are reconstituted for IL-5 production in the lungs, the challenged animals do have airway eosinophilia and bronchial hyperreactivity with allergen challenge. Furthermore, when mice are engineered to constitutively express IL-5 in their airways, airway eosinophilia and bronchial hyperreactivity develops, even in the absence of allergen challenge.⁹⁷ Conversely, when mice are treated with anti-IL-5 antibodies, they behave as IL-5–deficient mice.⁹⁸

Confounding these data are the limited studies in human subjects wherein the administration of anti-IL-5

antibodies to asthmatic patients substantially reduced peripheral blood eosinophilia but did not significantly affect airway hyperreactivity.⁹⁹ Subsequent studies have suggested that such treatment was not sufficient to reduce the eosinophils in the lung tissue.¹⁰⁰ One explanation for the observed results is that eosinophils might be more important in the tissue damage and airway remodeling that occurs over time in asthma.⁹² Because the animal models are acute models of airway inflammation and the human subjects who were studied had chronic asthma, differences in response to neutralization of IL-5 might also reflect the different stages of disease.⁹² More comprehensive studies with anti-IL-5 therapeutics will be needed to determine potential efficacy.

A potential role for eosinophils and IL-5 in mammalian reproduction has also been reported.^{101,102} By using an IL-5 transgene in mice, a direct regulatory role for eosinophils on ductal morphogenesis during postpubertal mammary gland development was demonstrated.¹⁰¹ However, the eosinophilia resulting from the IL-5 transgene did not have long-term consequences for adult reproductive function. Furthermore, in IL-5 null mice the absence of maternal and fetal IL-5 has no discernable effects on pregnancy and parturition.¹⁰²

Rarely, patients with an IL-5–producing T-cell leukemia or lymphoma present with eosinophilia.¹⁰³ This diagnosis should be considered in patients being evaluated for unexplained hypereosinophilia.

Although intuitively IL-5 and the consequent production of eosinophils seem crucial for immunity against parasites, there is limited evidence in mice that a lack of IL-5 results in a substantially increased risk for parasitic infection.^{104,105} Likewise, a lack of IL-5 and eosinophils is not associated with developmental abnormalities or an increased risk for malignancies.^{90,91} In summary, IL-5 is necessary for eosinophilic inflammation. However, its role in the pathogenesis of diseases associated with eosinophilic inflammation remains to be elucidated.

GM-CSF

The GM-CSFR α is expressed on CD34⁺ progenitor cells and all myeloid lineages (Fig 6), as well as some myeloid leukemias and vascular endothelial cells.⁷⁶ GM-CSF is secreted by activated T lymphocytes, eosinophils, mast cells, basophils, macrophages, endothelial cells, and bone marrow stromal cells. In addition to the promotion of myeloid differentiation,^{76,106} GM-CSF is a potent inhibitor of CD34⁺ progenitor cell differentiation into lymphoid progenitors or type 2 dendritic cells (Fig 6).¹⁰⁷ Likewise, GM-CSF inhibits the terminal differentiation of mast cells (Fig 6) and decreases Fc ϵ R1 expression and tryptase production by mature mast cells.¹⁰⁸ Recombinant human GM-CSF has been used clinically to promote myeloid cell recovery in myelosuppressed patients and is currently under investigation as an adjuvant for vaccines.¹⁰⁹ The in vitro inhibition of mast cell differentiation by GM-CSF also led to the use of GM-CSF as a treatment of systemic mastocytosis, although with limited benefit.¹¹⁰

When the gene encoding β c or the GM-CSF gene was deleted in mice, these animals had normal numbers of hematopoietic stem cells and progenitor cells but failed to accelerate production of granulocytes, macrophages, and eosinophils when challenged with infectious and inflammatory stimuli.^{11,111,112} However, the GM-CSF null mice also revealed a totally unexpected phenotype and elucidated a new role for GM-CSF.¹¹¹ These mice had PAP, which is characterized by abnormal surfactant metabolism. A similar PAP phenotype occurred when the gene encoding β c was deleted.^{111,112} In the GM-CSF null or β c null mice, as well as in human PAP, there is an accumulation of phospholipids and surfactant proteins within alveolar spaces. Recently, neutralizing autoantibodies against GM-CSF were detected in adult patients with PAP,¹¹³ and disease activity appears to correlate with the anti-GM-CSF antibody titer.¹¹⁴ These data, together with those of the gene knock-out studies, strongly suggests that PAP is caused by a lack of GM-CSF function, which is likely to be important for modulating pulmonary alveolar macrophage metabolic activity.

GM-CSF has also been implicated in cellular transformation.^{115,116} Expression of both GM-CSFR α and β c has been detected in different types of malignancies, indicating that GM-CSF can potentially transduce proliferative and survival signals to these transformed cells.¹¹⁵ Examples of these malignancies include chronic myeloid leukemia, acute myeloid leukemia, juvenile myelomonocytic leukemia, small-cell lung carcinoma, melanoma, certain breast cancer cell lines, and prostate cancer. The transient improvement of a patient with a GM-CSF–responsive myelomonocytic leukemia treated with a recombinant GM-CSF that had been mutated at glutamate 21 (Fig 2) to prevent β c signaling¹¹⁷ suggests the potential to treat such diseases with GM-CSF antagonists or GM-CSFR α antagonists. GM-CSF has also been shown to be involved in angiogenesis through the JAK2/STAT3 pathway in vivo.¹¹⁸ By using chicken chorioallantoic membranes, GM-CSF stimulation induced vessel sprouting from aortic rings without recruitment of inflammatory cells. Taken together, these data indicate that unregulated GM-CSF signaling can have physiologic consequences leading to the promotion of malignant growth.

Thus as with each of the β c–signaling cytokines, GM-CSF affects multiple leukocyte lineages, but its effects are quite distinct. Further delineation of the diverse biologic activities of GM-CSF should hold promise for the development of a new generation of biologic modifiers for use in medicine.

CONCLUSIONS AND FUTURE DIRECTIONS

IL-3, IL-5, and GM-CSF are emerging as cytokines with much broader functional activities than originally described, and it is likely that in the future yet-undefined regulatory functions and disease states will be linked to the β c–engaging cytokines. Current research in the field is focused on identifying specific signaling pathways acti-

vated by each cytokine, dissecting the molecular mechanisms that regulate signals elicited by these cytokines, and developing strategies to use these cytokines for modulating immune responses in conditions as diverse as malignancies, infections, PAP, and allergic inflammation.

In considering the composite contribution of IL-3, IL-5, and GM-CSF to immune responses, emerging knowledge on the biology of these cytokines should lead to a better understanding of the mechanisms of allergic inflammation. For example, the increased production of GM-CSF in allergic inflammation might reflect a complex regulatory mechanism whereby mast cell function and number is reduced, the phagocytic myeloid cells are increased, and the differentiation of type 2 and type 1 dendritic cells with the ability to promote T_H2 responses is decreased. Conversely, IL-3 promotes mast cell and basophil survival and function and promotes the differentiation of type 2 and type 1 dendritic cells with the ability to induce T_H2 responses, whereas IL-5 induces the differentiation and function of eosinophils. Future studies will determine whether selective modulation of IL-3, IL-5, and GM-CSF is an effective treatment for allergic inflammation or other diseases mediated by the β c-engaging cytokines.

The expert secretarial assistance of Anna Wirt and Tammy Kocurek in preparing the manuscript is greatly appreciated. The Biology of Inflammation Center has been selected as a Center of Excellence by the Federation of Clinical Immunology Societies (FOCIS Center of Excellence).

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