Molecular Pathways

Molecular Pathways: Interleukin-15 Signaling in Health and in Cancer

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Abstract

Interleukin-15 (IL-15) is a proinflammatory cytokine involved in the development, survival, proliferation, and activation of multiple lymphocyte lineages utilizing a variety of signaling pathways. IL-15 utilizes three distinct receptor chains in at least two different combinations to signal and exert its effects on the immune system. The binding of IL-15 to its receptor complex activates an "immune-enhancing" signaling cascade in natural killer cells and subsets of T cells, as well as the induction of a number of proto-oncogenes. Additional studies have explored the role of IL-15 in the development and progression of cancer, notably leukemia of large granular lymphocytes, cutaneous T-cell lymphoma, and multiple myeloma. This review provides an overview of the molecular events in the IL-15 signaling pathway and the aberrancies in its regulation that are associated with chronic inflammation and cancer. We briefly explore the potential therapeutic opportunities that have arisen as a result of these studies to further the treatment of cancer. These involve both targeting the disruption of IL-15 signaling as well as IL-15–mediated enhancement of innate and antigen-specific immunity.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

CME Staff Planners' Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

Upon completion of this activity, the participant should have a better understanding of IL-15 signaling in physiologic and pathologic conditions and the potential therapeutic development targeting this signaling pathway in inflammation and cancer.

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Background

Cytokines play a critical role during the host's immune response against infectious pathogens and malignant transformation. One such cytokine, interleukin-15 (IL-15), is central to the development, survival, and activation of natural killer (NK), T, and B cells (1–5). Discovered in 1994, IL-15 is a member of the "four α-helix bundle" cytokine family that signals via the common γ chain and the IL-2 receptor (IL-2R)-β chain, and as a result the two cytokines share select biologic functions (6–8). Here, we discuss the structure, regulation, and biologic functions of IL-15 in a wide variety of cell lineages as well as its role in genesis of cancer.

The human and mouse IL-15 gene have approximately 73% sequence homology and are mapped on chromosome 4 and 8, respectively (9). The DNA sequence of the human IL-15 gene consists of six protein-coding exons and five introns compared with eight exons and seven introns in the mouse (9, 10). The presence of two different signal peptides in the IL-15 gene results in alternative splicing and the subsequent generation of two IL-15 isoforms in both human and mouse (11). Although both the long signal peptide (LSP) and short signal peptide (SSP) isoforms produce mature proteins, they each have distinct intracellular trafficking, localization, and secretion patterns (11, 12). The LSP isoform is primarily located in the Golgi apparatus, early endosomes, and endoplasmic reticulum

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and is often secreted from the cell as a soluble protein. The SSP isoform is confined to the cytoplasm and nucleus, suggesting its role as a transcriptional regulator (11–16).

IL-15 transcript is abundantly produced by a large variety of tissues and cell types: (i) tissues include the placenta, skeletal muscle, kidney, lung, and heart tissue; and (ii) cell types include epithelial cells, fibroblasts, keratinocytes, nerve cells, monocytes, macrophages, and dendritic cells (6, 17–20). Transcriptional activation of IL-15 occurs via the binding of NF-kB and IRF-E to the 5′ regulatory region of IL-15, among other active motifs such as GC-binding factor (GCF), myb, and INF2 (20–26). Despite the abundant expression of IL-15 transcript, IL-15 protein is stringently controlled and expressed primarily within monocytes, macrophages, and dendritic cells (6, 17, 18). This discrepancy between IL-15 transcript and protein expression is due to complex translation and intracellular protein trafficking culminating in barely detectable levels of the protein in vivo. IL-15 posttranscriptional checkpoints include a complex 5′-untranslated region (UTR) containing (i) multiple AUG sequences upstream of the initiation codon; (ii) a C-terminal negative regulatory element; and (iii) an inefficient signal peptide (12, 14, 17, 23, 27). Collectively, these mechanisms serve to limit IL-15 protein production and secretion from its vast stores of transcript.

Despite the lack of homology in the amino acid sequence between IL-15 and IL-2, the mature IL-15 protein binds to the IL-2Rαβγ heterodimer, activating the intracellular signal leading to cell activation (6, 7, 28, 29). The third component of the IL-15R complex is a unique ε-chain (IL-15Rε). In contrast with the IL-2Rαβ chain that binds IL-2 with low affinity and confers high affinity for IL-2 only when noncovalently linked the IL-2Rβγ complex, IL-15Rε is by itself a high-affinity receptor for IL-15 (30). Once IL-15 is secreted out of the cell, it binds to either the membrane bound or the soluble form of IL-15Rε and is presented in trans to and bound by the IL-2Rβγ complex expressed on nearby effector cells to initiate cellular activation (31).

IL-15 utilizes select Janus-associated kinases (JAK) and signal transducer and activator of transcription (STAT) proteins as a means of initiating signal transduction for cellular activation (32). In lymphocytes, binding of IL-15 to the IL-2/15Rβγ heterodimer induces JAK1 activation that subsequently phosphorylates STAT3 via the β-chain and JAK3/STAT5 activation via its γ-chain (refs. 33, 34; Fig. 1). Phosphorylated STAT3 and STAT5 proteins form heterodimers that then translocate to the nucleus, where they activate transcription of the antipapoptotic protein bcl-2 and Mcl-1 (36).

Akt is activated via a phosphoinositide 3-kinase (PI3K)-dependent pathway, and in lymphocytes, this occurs despite the absence of PI3K-binding sites on the IL-2/15Rβγ (41, 42). The signaling mechanism utilizes an adaptor protein, Shc, which binds to a phosphotyrosine residue on the IL-2/15Rβ, resulting in activation of Grb2 and onto AKT via the Shc→Grb2→Gab2→PI3K→Akt signaling pathway to increase cell proliferation and/or survival (ref. 41; Fig. 1). In a third signaling pathway that follows the trans-presentation of IL-15 to IL-2/15Rβγ and Shc-mediated activation of Grb2, the latter binds to the guanine nucleotide exchange factor SOS to form a Grb2–SOS complex that then activates the Ras–Raf pathway by facilitating the removal of GDP from a member of the Ras subfamily that in turn activates the mitogen-activated protein kinase (MAPK) pathway for cellular proliferation (Fig. 1; refs. 43, 44). Thus, IL-15–mediated Grb2 phosphorylation regulates both the PI3K and MAPK pathways. Collectively, these signaling mechanisms induce expression and activation of downstream effector molecules such as c-myc, c-fos, c-jun, Bcl-2, and NF-kB (36).

In contrast with lymphocytes, mast cells express a distinct receptor, termed IL-15Rα, to activate the JAK2/STAT5 pathway (45). Murine mast cells treated with IL-15 seem to engage the IL-2/15Rγ chain to induce rapid phosphorylation of Tyk2/STAT6 for initiation of a Th2 type immune response (ref. 46; Fig. 1). In neutrophils, IL-15 has been shown to upregulate the antipapoptotic gene Mcl-1 through the MAPK pathway (47, 48).

Functionally, IL-15 supports cell expansion and maintenance by (i) inducing strong proliferative signals via JAK/STAT1 and Ras/MAPK signaling pathways and (ii) preventing cell death by increasing antipapoptotic proteins Bcl-2 and Bcl-XL, as well as decreasing proapoptotic proteins Bim and Puma through activation of the PI3K pathway (24, 32, 33, 36, 43, 44). In addition, IL-15 enhances the cytotoxic effector functions of lymphocytes by increasing the production of a cytokytic pore forming protein, perforin, and death-inducing enzymes, granzymes A/B, through all three pathways (39, 40, 49). IL-15 signaling is also well known to evoke a Th1 immune response by inducing release of IFN-γ and TNF-α; however, it can also trigger a Th2 response through release of IL-4 and IL-5 in activated human T cells (50, 51). Similarly, in mast cells and monocytes, IL-15 induces the release of IL-4 and the chemokine IL-8, respectively (46, 52). In addition to increasing expression of chemokine receptors in lymphocytes, IL-15 is a potent chemoattractant, thus inducing infiltration of T and NK cells at the site of its production (53, 54).

**Clinical–Translational Advances**

**Targeting IL-15 in cancer**

The antitumor effect of IL-15 on the immune system has been well documented in experimental systems (55); nonetheless, accumulating evidence suggests that IL-15 can also initiate and promote certain types of malignancies.

Multiple myeloma is a disease characterized by the accumulation of malignant plasma cells in the bone marrow and is particularly sensitive to IL-15 signaling. Exploring expression patterns of the IL-15R subunits in six multiple myeloma cell lines, as well as in the neoplastic cell fraction of 14 patients with multiple myeloma, Tinnofer and colleagues found that malignant plasma cells expressed all three components of the IL-15R heterotrimer (56). Although healthy B cells from normal donors downregulate...
IL-15Rα in response to IL-15, multiple myeloma cells do not exhibit such a reduction in response to IL-15 stimulation. In vitro, IL-15 overexpression in malignant plasma cells protects them from spontaneous apoptosis as well as a broader range of activation-induced cell death (56). These data suggest that multiple myeloma cells can inhibit apoptosis and sustain themselves via autocrine IL-15 stimulation, thereby becoming less dependent upon their microenvironment. Further studies, however, are needed to elucidate the cellular mechanisms of IL-15-mediated signaling in multiple myeloma pathogenesis.

IL-15 is a growth and viability factor for malignant T cells in cutaneous T-cell lymphoma (CTCL), a lymphoproliferative disorder characterized by migration and expansion of
malignant CD4^+ T cells in the skin (57). Skin lesion and peripheral blood T cells of patients with CTCL show overexpression of IL-15 mRNA and protein (57, 58). Although not yet directly proven, IL-15 is thought to play an important role in the epidermotropism found in CTCL, given its aberrant expression in the skin of these patients and its strong chemotactic properties for T cells (57, 59). IL-15 expression data from patients with CTCL strongly support the notion that in the early stages of CTCL, survival of malignant CD4^+ T cells is dependent on IL-15 supplied from the microenvironment, but as the disease progresses, malignant cells may sustain their own growth through autocrine IL-15 production and signaling. More importantly, IL-15–mediated JAK1 and JAK3 phosphorylation results in constitutive STAT activation that contributes significantly to the growth and survival of malignant T cells in patients with CTCL (60, 61). Of note, exposure of CD4^+ CTCL cells to IL-15 results in increased expression of antiapoptotic bcl-2 via the upregulation of STAT5 and c-myb, suggesting a profound role of IL-15 in LGL leukemia. Patients with leukemia of large granular lymphocytes (LGL) show increased serum levels of soluble IL-15Rα and constitutive expression of the IL-2/15Rβγ and membrane-bound form of IL-15 in leukemic blasts (64, 65). These data, and the fact that IL-15 is critical for the development and survival of both normal LGL (5, 66) and their malignant counterparts (67), support a role of IL-15 in LGL leukemia. Notably, two human LGL cell lines established from patients with CD3^- LGL leukemia show requirement of IL-2 or IL-15 signaling via the IL-2/15Rβγ for survival and proliferation in vitro (68, 69). While short-term exposure of IL-15 causes enhanced proliferation, cytokine production, and cytotoxic functions in normal LGLs (32–34, 39, 40), chronic IL-15–mediated activation via the JAK/STAT pathway, especially STAT3 and STAT5, can be leukemogenic. Somatic mutations in the SH2 domain of STAT3 have been discovered in the majority of patients with CD3^- LGL leukemia and 30% in NK-LGL leukemia patients (70, 71). Unprecedented in the cancer genome, a novel somatic mutation in the STAT3b gene has been discovered in 2% of patients with aggressive LGL leukemia (72). Because IL-15 signaling and STAT3/STAT5b somatic mutations increase transcriptional activity of STAT proteins, the evidence suggests that the IL-15 signaling pathway is critical for the genesis of LGL leukemia. Indeed, transgenic mice engineered to overexpress IL-15 develop spontaneous T-LGL and NK-LGL leukemia that exhibits hallmarks of the human disease (73, 74). More importantly, chronic exposure to IL-15 alone is sufficient to initiate malignant transformation of wild-type mouse LGL through two distinct pathways, both of which are regulated by IL-15–mediated induction of Myc (75). In the first cascade, IL-15 mediates Myc-induced overexpression of aurora kinase A and B, resulting in centrosome amplification and consequent chromosomal instability. In the second pathway, Myc induces the downregulation of microRNA (miR)-29b, which in turn increases the expression of DNA methyltransferases and the methylation of genomic DNA, furthering chromosomal instability and silencing key tumor suppressor genes (75).

Proteasome inhibition by bortezomib impairs the miR-29b–mediated signaling cascade by inhibiting binding of the "Myc/NEF–κB/Hdac1" corepressor complex at the miR-29b promoter (76). It is noteworthy that IL-15 reduces expression of the proapoptotic protein "Bid" in LGL leukemia via a proteasome-dependent mechanism, thereby protecting malignant cells from apoptosis, which can be reversed by blocking both IL-15 and IL-15Rα. In human LGL leukemic cells, induction of Bid by the proteasome inhibitor bortezomib increased leukemic cell death, suggesting that this could be an effective treatment option for this disease (77). Furthermore, in vivo administration of a novel formulation of bortezomib cured this otherwise fatal malignancy in mice with late stages of the disease (75), thus offering a new approach to treating patients with aggressive LGL leukemia.

Another therapeutic approach has been to use the monoclonal antibody Mikβ1 to block the presentation of IL-15 to the IL-2/IL-15Rβγ receptor, thereby inhibiting proliferation of an IL-15–dependent cell line, Kit-225, in vitro. Though successful in vitro, clinical trials with Mikβ1 antibody (both mouse and humanized antibody) have thus far not produced notable clinical responses in patients with LGL leukemia (78, 79). In a recent phase I clinical trial, 9 CD3^-CD8^-CD122^- T-LGL leukemic patients were treated with a single intravenous dose of 0.5, 1.0, or 1.5 mg/kg of Mikβ1 (3 patients/group), and these patients showed neither antibody associated toxicity nor clinical response (78). A phase I–II clinical trial utilizing the same antibody in patients with fatal HTLV-1–associated T-cell leukemia is ongoing (80). Finally, as a variety of STAT3 inhibitors come forth to the clinic, it will be important to screen LGL leukemia patients for STAT3 mutations for inclusion in early-phase clinical studies (81).

**IL-15 in clinical cancer therapy**

Early trials in several solid tumors are showing remarkable clinical responses in patients who are treated with agents that block negative regulators of T-cell activation, i.e., CTLA-4 and PD-1 (82–84). Likewise, transplantation of haploidentical, KIR-ligand mismatched, T-cell–depleted stem cells to patients with acute myeloid leukemia has yielded promising results (85). In both instances, it is...
likely that the critical effector lymphocyte populations (i.e., cytotoxic T cells and NK cells, respectively) are activated by IL-15, which is now being investigated in several phase I clinical trials as a single agent (80, 86–90). Thus, once proper dosing and delivery schedule are achieved with IL-15, a combination with these immunologic checkpoint inhibitors will hopefully be investigated to further improve response rates without exacerbating autoreactivity against nonmalignant tissues. The soluble IL-15Ra/IL-15 dimer is also in clinical development and might offer enhanced pharmacodynamic and pharmacokinetic properties over IL-15 alone (91). In contrast with IL-2, IL-15 does not seem to expand regulatory T cells that exert an immunosuppressive effect (92–94). Experimental studies comparing the two cytokines in vivo would suggest a significant difference in the antitumor potency mediated by T cells that favors IL-15 (55).

Conclusions

IL-15 is an important cytokine in the regulation of the normal host immune response and thus likely has a role in protection against pathogens and malignant transformation. The molecule utilizes a variety of signaling pathways to control lymphocyte development, survival, proliferation, and activation. Chronic stimulation can lead to malignant transformation of T and NK cells in experimental systems, and clinical data from patients with CTCL, HTLV-1, and LGL leukemia seem to support its oncogenic properties. Harnessing IL-15’s powerful properties to enhance lymphocyte effector function in the setting of malignancy is likely to take shape over the next decade, leading to its broad use in the treatment of both hematologic and solid tumor malignancies.

Authors’ Contributions

Writing, review, and/or revision of the manuscript: A. Mishra, M.A. Caligiuri
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Sullivan

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