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The shared and contrasting roles of interleukin-2 (IL-2) and IL-15 in the life and death of normal and neoplastic lymphocytes: implications for cancer therapy

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Abstract

Interleukin-2 (IL2) and IL15, members of the 4α-helix bundle family of cytokines, play pivotal roles in the control of the life and death of lymphocytes. Although their heterotrimeric receptors have two receptor subunits in common these two cytokines have contrasting roles in adaptive immune responses. The unique role of IL2 through maintenance of fitness of regulatory T cells (Treg) and activation-induced cell death (AICD) is the elimination of self-reactive T cells to prevent autoimmunity. In contrast to IL2, IL15 is dedicated to the prolonged maintenance of memory T-cell responses to invading pathogens. Blockade of IL2 and IL15 using monoclonal antibodies has been reported to be of value in the treatment of patients with leukemia, autoimmune disorders and in the prevention of allograft rejection. IL2 has been approved by the FDA for the treatment of patients with malignant renal cell cancer and metastatic malignant melanoma. Clinical trials involving recombinant human IL15 given by bolus infusions have been completed, and by subcutaneous and continuous intravenous infusions are underway in patients with metastatic malignancy. Furthermore, clinical trials are being initiated that employ the combination of IL15 with IL15Rα$^{+/-}$ IgFc.

Introduction

The immune system is dedicated to a series of goals including the generation of a rapid innate and adaptive immune response to invading pathogens, the elimination of autoreactive T cells to generate tolerance to self, and the maintenance of specific memory responses to pathogens. Such immune responses are normally regulated by cytokines. The cytokines that share the common gamma-chain (γc) among their receptor subunits including interleukin-2 (IL2), IL4, IL7, IL9, IL15 and IL21, play dominant roles in the regulation of immune responses (1, 2). Interleukin-2 and interleukin-15 have particularly pivotal roles in the control of the life and death of lymphocytes (3). In addition to the common γc, the heterotrimeric receptors for IL2 and IL15 share another subunit referred to as IL2/IL15Rβ

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(also known as IL2Rβ, CD122) (4, 5). Furthermore, the high-affinity forms of IL2R and IL15R contain a third cytokine-specific receptor α subunit IL2Rα (CD25) or IL15Rα (CD215), respectively (6, 7) (Figure 1). Additional structural data showed that the signaling complexes they form are topologically nearly identical (8). In light of the common receptor components and the fact that IL2 and IL15 signaling pathways also share JAK1 (Janus Kinase 1), JAK3 and STAT3/5 (signal transducer and activator of transcription 3 and 5) molecules, it was assumed that IL2 and IL15 would have similar functions. Indeed both cytokines stimulate the proliferation of T cells, induce the generation of cytotoxic T lymphocytes (CTL) and facilitate the maintenance of natural killer (NK) cells (3, 9-13). However in many adaptive immune responses IL2 and IL15 have distinct roles (Table 1). IL2 through its role in activation-induced cell death (AICD) and in the maintenance of fitness of regulatory T cells (Treg) is involved in the elimination of self-reactive T cells and thereby the prevention of autoimmune diseases (14). In contrast IL15 is critical for the maintenance of long-lasting, high-avidity T-cell responses to invading pathogens, a function that it achieves by supporting the survival of CD8 memory T cells (15, 16). This Masters of Immunology primer focuses on the distinct contributions of these cytokines to the regulation of the immune response. It also emphasizes efforts to translate insights concerning the biology of these cytokines into novel IL2- and IL15-mediated approaches to the treatment of cancer as well as to the opposite goal that employs antibodies to the cytokine receptors to treat cytokine-dependent malignancies and autoimmune diseases.

Genomic organization of IL2 and IL15 and control of gene expression

The genes encoding IL2 and IL15 are located on chromosomes 4q26-27 and 4q31, respectively (7). The cytokines are short-chain α-helical bundle type 1 cytokines with that of IL2 involving four exons and IL15 eight exons. IL2 synthesis is controlled by several mechanisms including silencing of the IL2 gene by B lymphocyte-induced maturation protein 1 (Blimp1) (13). Following T-cell interaction with mitogen or antigen-MHC complexes and dendritic cells IL2 synthesis is regulated at the level of transcription predominantly by CD4 cells and to a lesser extent by CD8 cells, NK cells and dendritic cells (DC) (13). IL15 transcription, translation and secretion are regulated through multiple complex mechanisms (17, 18). IL15 and IL15Rα proteins are co-expressed simultaneously predominantly by activated monocytes and DCs (3, 13). The transcription of the heterodimer IL15/IL15Rα occurs following the interaction of monocytes/DCs with type 1 or type 2 interferons (IFN), CD40 ligation or agents that act through Toll-like receptors (TLR) that activate NF-kB. Moreover IL15/IL15Rα protein expression is predominantly controlled at the levels of translation and secretion (17, 18). Three checkpoints have been identified that impede IL15 expression including multiple start codons (AUG) in the 5’ untranslated region (UTR), an unusually long signal peptide (48 amino acids) and a negative regulator near the C terminus of the precursor proteins (17, 18). The systemic elimination of these three checkpoints, including the removal of upstream AUGs, the replacement of the endogenous human IL15 leader with that of IL2, and the fusion of the C-terminus of the IL15 mature protein with a FLAG-epitope tag augmented the synthesis and secretion of IL15 250-fold.

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IL2 and IL15 receptor complexes and signaling

Three different IL2R complexes exist (7, 10, 12, 13, 19). The isolated IL2Rα subunit that is transiently expressed following T-cell receptor (TCR) activation or by contact of IL2 with the other subunits binds IL2 with low affinity (dissociation constant $K_d 10^{-8}$ M) without transducing a signal. The heterodimeric IL2Rβγc bind IL2 with intermediate affinity ($K_d 10^{-9}$ M), while the heterotrimeric IL2Rαβγc bind IL2 with high affinity (K_d 10^{-11} M). Both the heterodimeric and heterotrimeric receptors signal. In contrast to IL2Rα, the isolated IL15Rα has a high affinity for IL15 (K_d 10^{-11} M) (6, 19). With the Kit 225 leukemic T-cell line it was demonstrated that the common γc, IL2/IL15Rβ, IL2Rα, IL15Rα as well as Class I and II MHC are associated elements in supramolecular receptor clusters in lipid rafts prior to the cytokine addition and that IL2 and IL15 compete for the use of γc (20). On addition IL2 initially binds to IL2Rα resulting in a structural change in the element of IL2 that binds to the β chain followed by joining with IL2/IL15Rβ and γc that increases the proximity of its receptor subunits (20). The binding of IL2 or IL15 to the IL2/IL15Rβ and γc heterodimer induces JAK1 activation via the β chain and JAK3 via the γ chain that together phosphorylate tyrosine on the cytokine receptors and induce the tyrosine phosphorylation of STAT3, STAT5A and STAT5B that via SH2 domain interactions homodimerize, translocate to the nucleus and bind to regulatory regions of target genes (3, 10, 20-24). Additional IL2 and IL15 signaling mechanisms include the adaptor protein Shc that binds to a phosphotyrosine residue on IL2/IL15Rβ resulting in the activation of Grb2 and Akt via the Shc, Grb2, Gab2, PI3K, PIP3, Akt, mTOR, p70, S6 signaling pathway (20-23). In a third signaling pathway IL2/IL15 signaling is associated with activation of SOS and Grb2 to form a Grb2/SOS complex that in turn activates the Ras, Raf, MEK, MAPK ERK pathway involved in cellular proliferation. Collectively these signaling pathways induce the expression and activation of c-myc, c-fos, c-jun, Bcl-2, Bcl-xL and NF-kB as well as decrease expression of proapoptotic Bim and PUMA (25). In addition to the positive signals, lymphocytes have evolved sophisticated mechanisms to prevent excessive responses to IL2 and IL15, including the induction of the expression of suppressors of cytokine signaling (SOCS) including SOCS1, SOCS3 and CIS as well as PIAS (26). SOCS proteins inhibit components of the cytokine signaling cascade via direct binding or by preventing access to the signaling complex. The SOCS proteins also target signal transducers for proteasomal destruction.

Distinct functions of IL2 and IL15

IL2 and IL15 have several similar functions as a consequence of their sharing of receptor components IL2/IL15Rβ and γc and their use of common JAK and STAT signaling molecules. These functions include stimulating the proliferation of activated $CD4^-CD8^-$ (double negative), $CD4^+CD8^+$ (double positive), $CD4^+$ and $CD8^+$ (single positive) T cells and their differentiation into defined effector T-cell subsets following antigen-mediated activation (3, 7, 10, 19). Furthermore, the two cytokines facilitate the production of cytotoxic T lymphocytes (CTL) and immunoglobulin synthesis by B cells that have been stimulated with IgM-specific antibodies or by CD40 ligation. IL2 and IL15 also stimulate the generation and proliferation of NK cells (27). In addition to these similarities, there are distinctions between the functions of IL2 and IL15 that are crucial in the homeostasis of adaptive immune responses. IL2 has paradoxical functions in T-cell homeostasis: IL2 acts as

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a T-cell growth factor during the initiation of an immune response but it has a crucial role in the termination of T-cell responses for the maintenance of self-tolerance. Although IL2 signals are not essential for Treg development in the thymus, they are critical for the maintenance of Tregs in the periphery (14, 28-31). IL2 and all three IL2 receptor chains (α, β and γ) are required for high-affinity IL2 binding for Foxp3 (forkhead box P3) expression (28). The transcription factor AML1 (acute myeloid leukemia with AE-1)/Runx1 (Runt-related transcription factor 1) activates IL2 and IFNγ gene expressions in conventional $CD4^+$ T cells through binding to their respective promoters (31). In natural Tregs Foxp3 interacts physically with AML1 (31). Several lines of evidence support a model in which this interaction suppresses IL2 and IFNγ production, upregulates Treg cell-associated molecules and exerts suppressive activity (28).

In contrast to IL2, IL15 has no major net effect on the maintenance of the fitness of Foxp3-expressing Tregs. IL2 and IL15 also have distinct roles in AICD (11, 32). IL2 is a critical determinant in the choice between proliferation and death. Both CD4 and CD8 T cells previously exposed to antigen and high-level of IL2 undergo apoptosis after persistent TCR stimulation in a process involving induction of the death receptor FAS (CD95) and FAS ligand (CD95 L32). In contrast, IL15 is an anti-apoptotic factor in several systems. In particular, in IL15-transgenic mice, IL2-induced AICD is inhibited (11). Furthermore, IL15 promotes the maintenance of $CD8^+CD44^{hi}$ memory T cells (15, 16).

These observations from *ex vivo* functional studies were supported by analysis of mice with disrupted cytokine or cytokine-receptor genes (33, 34). IL2-, IL2Rα- and IL2/IL15Rβ-deficient mice developed a marked enlargement of peripheral lymphoid organs that was associated with polyclonal expansions of T- and B-cell populations, a dysregulated proliferation that reflects the impairment of Treg cell-fitness and AICD (33). IL2Rα-deficient mice develop autoimmune diseases such as hemolytic anemia and inflammatory bowel disease. In contrast mice that are deficient in IL15 or its private receptor IL15Rα do not develop lymphoid enlargement, increased serum immunoglobulin concentrations or autoimmune disease (34). Rather such mice have a marked reduction in the number of thymic and peripheral NK cells, natural killer T (NKT) cells, γδ T cells and intestinal intraepithelial lymphocytes (IEL). Furthermore, IL15Rα-deficient mice show a marked reduction in $CD8^+$ $CD44^{hi}$ memory T cells.

How do IL2 and IL15 with two receptor subunits and the JAK/STAT signaling pathway in common manifest distinct functions?

One factor underlying the distinct functions of IL2 and IL15 is that the unique receptor components, i.e. the two α chains, are differentially distributed. IL2Rα is mainly expressed by activated T and B cells, whereas IL15Rα is predominantly expressed by activated monocytes and DCs (3, 19, 35). In addition to the signaling pathways they have in common, there are receptor-signaling pathways that distinguish the two cytokines (35, 36). In particular, IL15 mainly induces T-cell proliferation through FKBP12 (FK506-binding protein 1A, 12kDA also known as FKBP1A)-mediated activation of p70S6 kinase ($p70^{S6K}$). By contrast FKBP12 is not required for proliferation induced by IL2. Furthermore, FKBP12-deficiency strongly affects the phosphorylation of extracellular signal-regulated

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kinase (ERK) and p70S6 kinase ($p70^{S6K}$) in response to IL15 but not to IL2 (36). Another protein FKBP12.6 has been found to be involved in the T-cell response to IL2 but not to IL15.

A most critical factor in the functional differences between IL2 and IL15 involves the fact that IL2 is a predominantly secreted molecule that in its soluble form or linked to extracellular matrix binds to preformed high-affinity heterotrimeric receptors at the surface of activated cells (37) (Figure 1). In contrast to IL2, IL15 is only secreted along with IL15Rα in small quantities and is mainly membrane-bound. IL15 induces signaling in the context of cell-cell contact at an immunological synapse. Stimulation of monocytes or DCs with type I or type II IFN together with activation of nuclear factor-kB (NF-kB) through ligation of CD40 or TLR4 with lipopolysaccharide induces the coordinate simultaneous expression of IL15 and IL15Rα. The IL15 and IL15Rα expressed by these monocytes and DCs then become associated on the cell surface and recycle through endosomal vesicles for many days resulting in persistence of membrane-bound IL15Rα and associated IL15 (37). As part of an immunological synapse IL15Rα presents IL15 *in trans* to cells that express IL2/IL15Rβ and γc but not IL15Rα (37-41). Such targets of IL15/IL15Rα trans-presentation include NK cells and CD8 memory T cells. In addition to the signals provided by IL15, co-stimulatory signals are transmitted between the two cells during the synapse cell-cell trans-presentation process. In parallel to IL15 early in the immune response CD25 on DCs can present IL2 *in trans* to antigen-specific T cells (42).

Furthermore in addition to its dominant trans-presentation, IL15 can also signal *in cis* to cells that express IL15Rα in addition to IL2/IL15Rβ and the common γc (43-45). In particular, Zanoni and coworkers demonstrated that IL15 *cis* presentation is required for optimal NK-cell activation in lipopolysaccharide-mediated inflammatory conditions (45). In addition to the classical heterotrimeric IL15 receptor, JAK1, JAK3, STAT3/5 pathway there are novel receptor-signaling transduction pathways for IL15 in mast cells (46). IL15 signaling in mast cells does not involve IL2/IL15Rβ or the common γc, rather it involves distinct receptors. Furthermore, mast cell IL15 receptors recruit JAK2 and STAT5 instead of JAK1/3 and STAT3/5 that are activated in T cells. Finally a number of groups have reported that in the absence of the common γc and independently of IL2/IL15Rβ and JAK/STAT signaling that IL15 can signal through IL15Rα, JNK and NF-kB to drive RANTES production by myeloid cells (47-49).

Disorders of the IL15/IL2/IL15 cytokine, receptor system

The gene encoding γc is mutated in individuals with X-linked severe combined immunodeficiency (SCID) who lack T cells and NK cells; B cells are present but nonfunctional (7, 50). Mutations of JAK3 downstream of γc are present in autosomal recessive SCID (51, 52). IL2, IL2α and IL2/IL15Rβ deficiencies are associated with polyclonal expansions of T- and B-cell populations and autoimmune diseases. In the absence of IL2 signals the number of Tregs declines markedly whereas the number of Th17 cells increases (7, 53). IL2/IL15Rβ-deficiency is also associated with the absence of NK cells secondary to defective IL15 signaling.

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Excessive dysregulated IL15 expression has been reported in patients with a range of autoimmune inflammatory diseases including rheumatoid arthritis, multiple sclerosis, ulcerative colitis, type 1 diabetes, psoriasis, and refractory celiac disease (3, 9). IL15, a proinflammatory cytokine, may precede the expression of TNFα and downstream cytokines IL1, IL6 and GMCSF (3). The retrovirus HTLV-1-encoded tax protein expressed in adult T-cell leukemia (ATL) and the neurologic disease HAM/TSP activates two autocrine and one paracrine system involving IL2/IL2Rα, IL15/IL15Rα and IL9 (54, 55). As a consequence of these stimulatory autocrine/paracrine loops, the ATL cells among *ex vivo* PBMCs proliferate spontaneously.

IL2R- and IL15R-directed immunotherapy

The specific IL2R subunit IL2Rα has been an exceptionally valuable target for immunotherapy (56-64). The scientific basis for this is that IL2Rα is not expressed by resting cells other than Tregs and $CD56^{hi}CD16^{lo}$ NK cells but is constitutively expressed by an array of malignant cells of various T- and B-cell leukemias as well as T cells involved in select autoimmune disorders and those that participate in organ allograft rejection (56, 59). One form of IL2 receptor-directed therapy involves the use of unarmed antibodies specific for IL2Rα including basiliximab (Simulect: Novartis AG) and daclizumab (also known as anti-Tac, Zenapax), the first humanized antibody that was approved by the FDA (56, 58, 60, 61). The administration of these antibodies blocks the interaction of IL2 with IL2R leading to cytokine deprivation and death of IL2-dependent cells. Treatment with daclizumab has provided effective therapy for patients with non-infectious uveitis (60). Furthermore treatment with daclizumab has resulted in an over 70% reduction in new-contrast enhancing lesions in patients with multiple sclerosis who had failed to respond to treatment with IFNβ (61). In addition, daclizumab was shown to provide effective therapy for select patients with human T-cell lymphotropic virus 1-associated (HTLV-1) ATL—a leukemia that has been viewed as the leukemic counterpart of Tregs (56, 64). In additional clinical trials daclizumab armed with toxins or β-emitting radionuclides that results in selective delivery of toxins or radionuclides to cells expressing IL2Rα has provided effective therapy for select patients with ATL and Hodgkin's lymphoma (62, 63).

An antibody directed toward IL2/IL15Rβ (Hu-Mik-Beta-1) has been shown to block the trans-presentation of IL15 but not the action of IL15 on *cis* expressed heterotrimeric IL15 receptors (44). Hu-Mik-Beta-1 is being evaluated in patients with T-cell large granular lymphocytic leukemia, the HTLV-1-associated neurologic disease HAM/TSP and in patients with refractory celiac disease (44). In patients with HTLV-1-associated disorders the protein encoded Tax activates two autocrine (IL2/IL2R, IL15/IL15R) and one paracrine (IL9) pathway that signals through the γc and the JAK1/3, STAT3/5 pathway (54, 55). In light of this multi- cytokine activation in lieu of a monoclonal antibody inhibition, utilizing a JAK inhibitor (e.g. ruxolitinib/tofacitinib) or more appropriately a JAK1-specific inhibitor could be of value (55).

IL2 in the therapy of cancer

Rosenberg and coworkers first utilized ultra-high doses of IL2 to treat patients with metastatic renal cell carcinoma (65). Such high-dose IL2 therapy resulted in a significant

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clinical response (around 15%); however it also resulted in very significant toxicity. The IL2 doses used amounted to ~50 million units per injection every 8 hours that resulted in plasma IL2 concentrations more than sufficient to saturate the high-affinity as well as the intermediate-affinity IL2 receptors. The end result was stimulation of a massive secondary release of proinflammatory cytokines IFNγ, IL6, TNFα and GM-CSF in concert with direct binding of IL2 to $CD25^+$ expressing endothelial cells which induced the vascular capillary-leak syndrome. IL2 (aldesleukin, Proleukin) was approved for the treatment of metastatic renal cell cancer and malignant melanoma. Despite its accepted role, IL2 has additional negative characteristics. IL2 has a short *in vivo* half-life. Furthermore IL2 has a dual role as an immunomodulator that stimulates proliferation of effector cells that kill cancer cells but, as noted above, also stimulates checkpoint cells that suppress the immune response by the maintenance of inhibitory $CD25^+$ $Foxp3^+$ Treg cells that are involved in AICD.

IL2 has also been used in ultra-low dose therapy based on the known affinity of IL2 heterotrimeric receptor components (19, 66). These trials were performed in patients with cancer or following bone marrow transplantation and resulted in selective expansion of $CD56^+$ $CD3^-$ $CD16^-$ NK cells based on the rationale that such resting cells have a constitutive expression of the high-affinity heterotrimeric IL2Rα, β, γc cells. The ability of low-dose IL2 to expand such NK cells with little or no toxicity has been confirmed in additional patients with cancer and/or immunodeficiency. However, low-dose IL2 therapy for cancer has been disappointing presumably due in part to the expansion of Tregs.

In addition to natural IL2, a conformational switch has been exploited to engineer an interleukin-2 'superkine' that allows tight binding to γc in the absence of IL2Rα. The super-IL2 had improved antitumor activity in mice bearing three types of human tumors (67). Furthermore, Boyman, Surh, Sprent and coworkers demonstrated that some IL2/anti-IL2 monoclonal body immune complexes caused massive (> 100-fold) expansion of $CD8^+$ T cells and activation of NK cells *in vivo* due to the markedly augmented activity of IL2 (68). Thus IL2 antibody complexes have been used to selectively boost the immune response and reduce tumor metastases (68-71). Furthermore, an antibody-interleukin-2 fusion protein has been shown to overcome tumor heterogeneity by induction of a cellular immune response (70). In parallel IL15/antibody-fusion proteins for cancer immunotherapy mimicking IL15 trans-presentation at the tumor site have been generated (72).

IL15 in the treatment of cancer

A number of studies in murine models in particular CT26 and MC38 colon adenocarcinoma, $P1A^+$, B-16 melanoma, and TRAMP-C2 prostatic cancer suggested that IL15 may prove to be of value in the therapy of neoplasia (3, 13, 19, 22, 73-82). Intravenous administration of murine IL15 enhanced survival of such tumor-bearing mice (74). Furthermore, Klebanoff and coworkers demonstrated that IL15 enhanced the *in vivo* activity of tumor-reactive $CD8^+$ T cells in the TCR transgenic mouse (pmel-1) whose $CD8^+$ T cells recognized an epitope derived from the melanoma antigen GP-100 (73). In addition, Bessard and coworkers demonstrated high antitumor activity of RLI, an IL15-IL15Rα fusion protein, in metastatic melanoma and colorectal cancer models (83). On the basis of the animal preclinical trials with IL15 great interest was generated among leading immunotherapeutic experts

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participating in the NCI Immunotherapy Agent Workshop that ranked IL15 as the most promising unavailable immunotherapeutic agent to be brought to human therapeutic trials (84).

The safety of IL15 was evaluated in rhesus macaques by Munger, Mueller, Berger, Lugli, Waldmann and Sneller (76, 85-89). A 12-day bolus of intravenous administration of 20 mcg/kg/day of IL15 to rhesus macaques was associated with a 4-fold to 8-fold increase in the number of circulating NK, stem, central and effector memory CD8 T cells (86). Subsequently alternative routes of administration were evaluated in rhesus macaques including continuous intravenous infusion (CIV) and subcutaneous (SC) administration of IL15. The administration of IL15 by CIV at 20 mg/day for 10 days led to a 10-fold increase in the number of circulating NK cells, a 15-fold increase in the number of circulating monocytes, and a massive 80-fold to 100-fold increase in the number of circulating effector memory CD8 T cells (88). Subcutaneous infusions at 20 mcg/kg/day for 10 days led to a more modest 10-fold expansion in the number of circulating effector memory CD8 T cells.

Clinical trials using IL15 in the treatment of cancer

Five clinical trials have been initiated using *Escherichia coli* rhIL15 in the treatment of cancer (90-93). The primary goal of the completed trial: a phase I study of recombinant human IL15 in adults with refractory metastatic malignant melanoma and metastatic renal cancer was to determine the safety, adverse event profile, dose-limiting toxicity and maximum tolerated dose of rhIL15 administered as a daily intravenous bolus infusion for 12 days to subjects with metastatic malignant melanoma or metastatic renal cell cancer (94). The study was initially planned as a phase I dose-escalation trial starting with an initial dose of 3 mcg/kg/day for 12 days. However after the initial patient developed grade 3 hypotension and another patient developed grade 3 thrombocytopenia the protocol was amended to add two lower doses 1.0 and 0.3 mcg/kg/day (94). Two of four patients given the 1.0 mcg/kg/day dose had persistent grade 3 ALT and AST elevations that were dose-limiting. All 9 patients with IL15 administered at 0.3 mcg/kg/day received all 12 doses without DLT. The maximum tolerated dose of rhIL15 was determined to be 0.3 mcg/kg/day.

There was a consistent temporal pattern of post-treatment adverse events in patients given the 3 mcg/kg/day dose of IL15 with fever and rigors beginning 2 ½ to 4 hours after the start of IL15 infusions, with a blood pressure drop to a nadir of approximately 20 mm/Hg below pretreatment levels 5 to 9 hours after the infusion. These changes were concurrent with a maximum of 50-fold elevations of circulating IL6 and IFNγ concentrations. Flow cytometry of peripheral blood lymphocytes revealed an efflux of NK and memory T cells from the circulating blood within minutes upon IL15 administration followed by influx and hyperproliferation leading to 10-fold expansions of NK and γδ T cells that ultimately returned to baseline. Furthermore, there were significant increases in the number of CD8 memory phenotype T cells. In this first-in-human phase I trial there were no responses with stable disease as the best response. However 5 patients manifested a decrease of between 10% and 30% of their marker lesions and 2 patients had clearing of lung lesions. Subsequently alternative dosing strategies including continuous intravenous infusions and subcutaneous infusions of IL15 in trials have been initiated (91, 92).

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IL15/IL15Rα

Although IL15 may show efficacy in the treatment of metastatic malignancy in human trials it has not been optimal when used as a single agent for cancer therapy. A particular challenge is that there is only a low level expression of IL15Rα on resting DCs (95). Indeed, the true IL15 cytokine may not be an IL15 monomer but rather may be considered as an IL15Rα/IL15 heterodimeric cytokine. Physiologically IL15 is produced as a heterodimer in association with IL15Rα. Furthermore in mice it is the heterodimer alone that is stably produced and transported to the surface of the cell (38, 39). On cleavage from the cell surface IL15Rα/IL15 elements are associated in the serum as the sole form of circulating IL15 (95). To address the issue of deficient IL15Rα, IL15/IL15Rα and IL15Rα IgFc have been produced and entered into clinical trials evaluating patients with metastatic malignancy (96-100).

As an alternative strategy, agents are available that induce IL15Rα expression on DCs that could be given in conjunction with IL15 to circumvent the problem discussed above with IL15 when used in monotherapy. The combination of IL15 with the agonistic anti-CD40 antibody FGK4.5 showed additivity/synergy in the MC38 murine model of colon cancer and the TRAMP-C2 model of prostatic cancer (74, 80). The administration of the anti-CD40 antibody was associated with an increased expression of IL15Rα on $CD11^+$ DCs. Furthermore, in the murine synergic tumor model treatment with IL15 with the agonistic anti-CD40 antibody alone significantly prolonged the survival of the TRAMP-C2 tumor-bearing mice. Moreover it was demonstrated that the combination of IL15 with anti-CD40 produced markedly additive effects when compared to either agent administered alone. The combination appeared to circumvent the problem of “helpless” CD8 T cells wherein the CD8 T cells produced are not tumor antigen-specific (74). The administration of either IL15 or anti-CD40 alone did not augment the number of tumor-specific tetramer-positive CD8 T cells in the TRAMP-C2 model system. However the administration of the combination of IL15 plus the agonistic anti-CD40 antibody was associated with a meaningful increase in the number of TRAMP-C2 tumor-specific SPAS-1/SNC9-H8 tetramer-positive CD8 T cells (74).

Agents to relieve checkpoints on the immune system to optimize IL15 action

As is true with other cytokines, IL15 is associated with the expression of immunologic checkpoints including the inhibitory cytokine IL10 and the expression of PD-1 on CD8 T cells. In addition, IL15 was shown to be critical in the maintenance of $CD122^+CD8^+$ negative regulatory T cells (101-102). To address the issue of induced checkpoints, IL15 was administered in combination with agents to remove these checkpoints with antibodies to cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death- ligand 1 (PD-L1) (81, 103). In the CT26 or MC38 colon carcinoma or the TRAMP-C2 prostatic cancer syngeneic tumor models IL15 alone provided modest antitumor activity. The addition of either anti-CTLA-4 or anti-PD-L1 alone in association with IL15 did not increase the action of IL15. However tumor-bearing mice receiving IL15 in combination with both anti-checkpoint antibodies together manifested a marked prolongation of survival (81, 103).

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Combination therapy with IL2 and IL15 with anticancer monoclonal antibodies to augment antibody-dependent cell-mediated cytotoxicity

The predominant approaches involving IL2 and IL15 are based on the hypothesis that the host is making an immune response, albeit inadequate, to their tumor and that this can be augmented by the administration of an IL2- or IL15-containing agent. However these cytokines could also be used in drug combinations where an additional co-administered drug provides the specificity directed toward the tumor. In particular, IL2 or IL15 could be used with anticancer vaccines, cellular therapy or with tumor-directed monoclonal antibodies (mAb) (83, 104-111). Given the capacity of IL2 and IL15 to increase the number of activated NK cells, monocytes and granulocytes, a very attractive antitumor strategy would be to use the optimal IL2 or IL15 agent and dosing strategy in conjunction with antitumor mAbs to augment their ADCC action (105-110). For example, when low-dose IL2 therapy with intermediate-dose boluses was combined with administration of R24, a murine mAb that recognizes a malignant melanoma antigen, three of the 18 evaluable patients generated clinical responses (108). In addition, IL2 has been given in combination with rituximab for the treatment of B-cell malignancies with varying success (110). An alternative strategy is the direct coupling of a tumor-specific mAb and a cytokine as a fusion protein. Such an agent has been generated that couples IL2 to tumor-specific mAbs (111-112). In parallel with an IL15 conjugate Vincent and coworkers reported highly potent anti-CD20-RLI immunocytokine targeting established human B-cell lymphoma in SCID mice (113). It is hoped that with the diverse approaches discussed IL15 and IL2 will take central places in the combination treatment of cancer.

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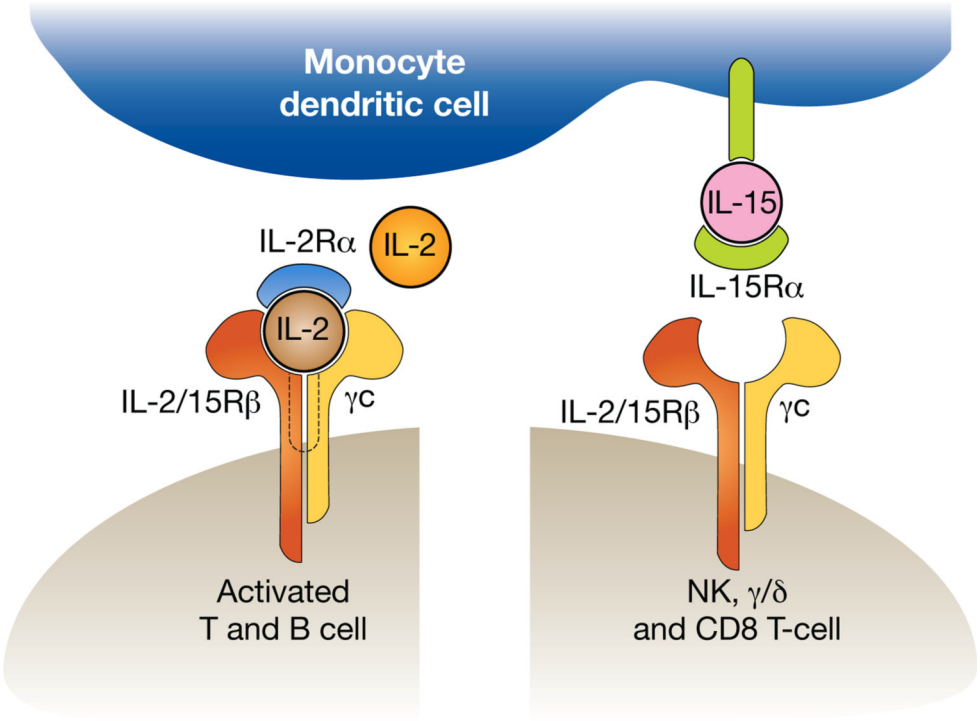

Figure 1. Mode of interaction of IL2 and IL15 with their receptors

IL2 and IL15 share the common γc and IL2/IL15Rβ chains. Furthermore, the high-affinity forms of IL2R and IL15R contain a third cytokine-specific receptor α subunit, IL2Rα or IL15Rα. IL2 is predominantly a secreted cytokine that binds to preformed high-affinity heterotrimeric receptors. By contrast, IL15 is a membrane-associated molecule that signals at an immunological synapse between antigen-presenting cells and CD8 T cells or NK cells. IL15Rα on the surface of activated monocytes or dendritic cells presents IL15 *in trans* to cells that express IL2/IL15Rβ and γc, thereby allowing signaling through these complexes.

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Table 1

Comparison of IL2 and IL15

Properties	IL2	IL15
Gene structure and location	Four exons, chromosome 4q26	Eight exons, chromosome 4q31
Main site of synthesis	Activated CD4 Th1 cells	Activated dendritic cells and monocytes
Mechanism of regulation of expression	Transcriptional regulation and stabilization of mRNA	Mainly post-transcriptional, during translation and intracellular trafficking
Receptor	Cis-presentation to IL2Ra, IL2/IL15RP and γc co-expressed on activated T and B cells	IL15Rα/IL15 on the surface of dendritic cells and monocytes trans-presented to NK cells and $CD8^+$ memory T cells expressing IL2/IL15Rβ and γc
Unique Function	Maintenance of Tregs and elimination of self-reactive T cells mediated by AICD to yield self- tolerance	Maintenance of NK cells and $CD8^+CD44^h$ memory T cells to provide a long-term immune response to pathogens
Features of mice deficient in gene-encoding cytokine or its private receptor α-chain	Marked enlargement of peripheral lymphoid organs, polyclonal expansion of T and B cells associated with autoimmune disorders	Marked reduction in number of NK, NKT gamma/delta and $CD8^+CD44^{hi}$ memory T cells

Data are from references 3, 7, 9, 13, 19, 22. Abbreviation: AICD=activation-induced cell death; yc=common gamma chain; IL2Rα=IL2 receptor alpha chain; IL2/IL15Rβ=β-chain of the IL2 and IL15 receptor; IL15Rα=IL15 receptor α-chain; Treg=$CD4^+CD25^+$ regulatory T cell