The Shared and Contrasting Roles of IL2 and IL15 in the Life and Death of Normal and Neoplastic Lymphocytes: Implications for Cancer Therapy ᶜᵐᵉ

Thomas A. Waldmann

Abstract

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 and activation-induced cell death is the elimination of self-reactive T cells to prevent autoimmunity. In contrast with 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 studies assessing subcutaneous and continuous intravenous infusions are under way in patients with metastatic malignancy. Furthermore, clinical trials are being initiated that employ the combination of IL15 with IL15Rα$^{+/-}$ IgFc. *Cancer Immunol Res; 3(3); 219–27. ©2015 AACR.*

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Editor's Disclosures

The following editor(s) reported relevant financial relationships. G. Dranoff—None.

CME Staff Planners' Disclosures

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

Learning Objectives

The cytokines interleukin-2 (IL2) and IL15 bind heterotrimeric receptors that share two common subunits. Both cytokines stimulate T-cell proliferation, induce the generation of CTLs, and facilitate the maintenance of natural killer cells. Through its role in maintaining the fitness of regulatory T cells and in activation-induced cell death (AICD), IL2 is involved in the elimination of self-reactive T cells and prevention of autoimmunity. IL15 inhibits AICD and prolongs CD8 memory T-cell responses. IL2 has been approved by the FDA for malignant renal cell cancer and metastatic melanoma. Clinical trials of IL15/IL15R are ongoing. Upon completion of this activity, the participant should gain a basic knowledge of the pivotal roles of the IL2 and IL15 cytokine/receptor system in controlling the life and death of lymphocytes and the current therapeutic utility of the IL2/IL15 cytokine/receptor systems.

Acknowledgment of Financial or Other Support

This activity does not receive commercial support.

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 IL2, IL4, IL7, IL9, IL15, and IL21, play dominant roles in the regulation of immune responses (1, 2). IL2 and IL15 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 (also known as IL2Rβ, CD122; refs. 4, 5). Furthermore, the high-affinity forms of IL2R and IL15R contain a third cytokine-specific receptor α subunit IL2Rα (CD25) or

Lymphoid Malignancies Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland.

Corresponding Author: Thomas A. Waldmann, National Cancer Institute, NIH, 10 Center Drive, Building 10, Room 4N115, Bethesda, MD 20892-1374. Phone: 301-496-6656; Fax: 301-496-9956; E-mail: tawald@helix.nih.gov

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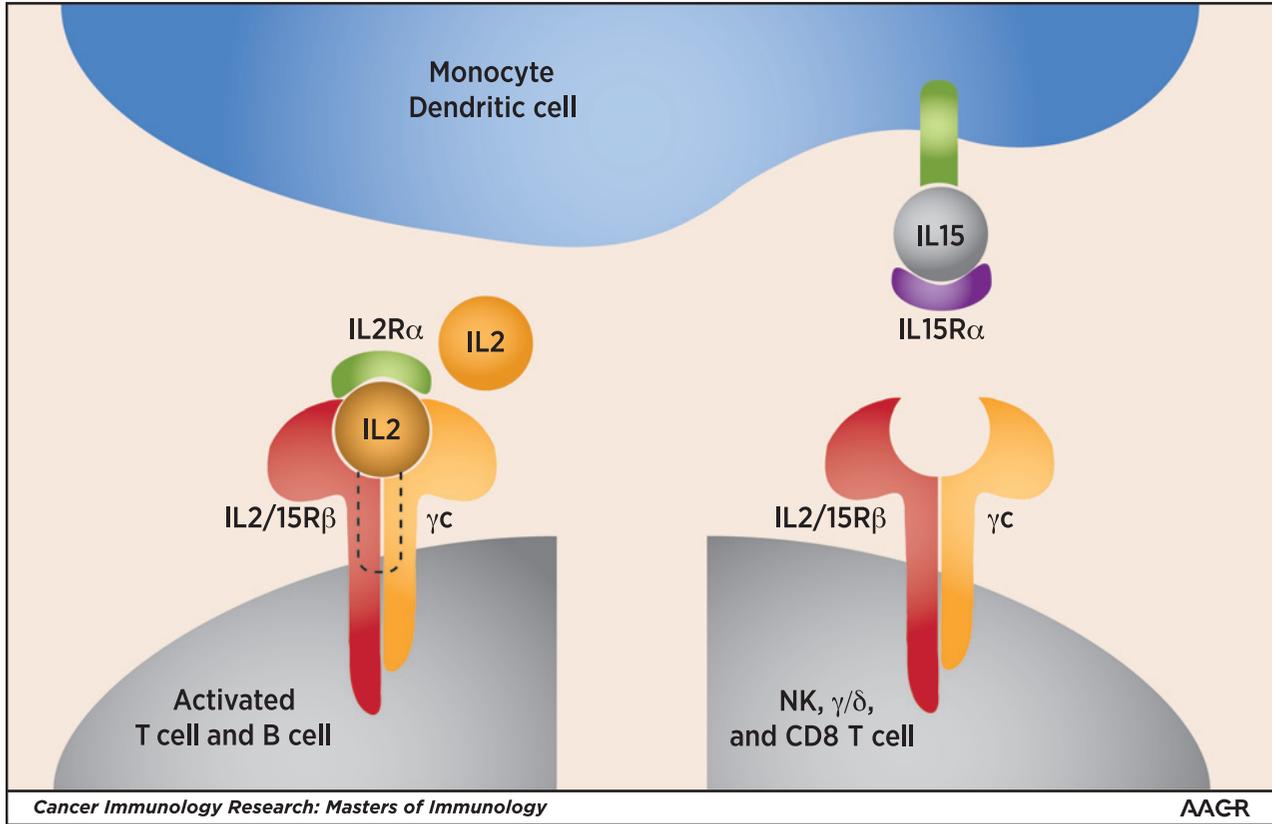*Cancer Immunology Research: Masters of Immunology*

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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 DCs presents IL15 *in trans* to cells that express IL2/IL15Rβ and γc, thereby allowing signaling through these complexes. Adapted from Waldman (3).

IL15Rα (CD215), respectively (refs. 6, 7; Fig. 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

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 DCs and monocytes
Mechanism of regulation of expression	Transcriptional regulation and stabilization of mRNA	Mainly posttranscriptional, during translation and intracellular trafficking
Receptor	*Cis*-presentation to IL2Rα, IL2/IL15Rβ, and γc coexpressed on activated T and B cells	IL15Rα/IL15 on the surface of DCs 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+CD44hi 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+CD44hi memory T cells

NOTE: Data are from refs. 3, 7, 9, 13, 19, 22.
Abbreviations: IL2Rα, IL2 receptor alpha chain; IL2/IL15Rβ, β chain of the IL2 and IL15 receptor; IL15Rα, IL15 receptor alpha chain; Treg, CD4+CD25+ regulatory T cell.

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 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; ref. 13). Following T-cell interaction with mitogen or antigen–MHC complexes and dendritic cells (DC), IL2 synthesis is regulated at the level of transcription predominantly by CD4 cells and to a lesser extent by CD8 cells, NK cells, and DCs (13). IL15 transcription, translation, and secretion are regulated through multiple complex mechanisms (17, 18). IL15 and IL15Rα proteins are coexpressed 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 IFNs, CD40 ligation, or agents that act through Toll-like receptors (TLR) that activate NF-κB. 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, 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.

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 $K_d = 10^{-8}$ mol/L) without transducing a signal. The heterodimeric IL2Rβγc binds IL2 with intermediate affinity ($K_d = 10^{-9}$ mol/L), whereas the heterotrimeric IL2Rαβγc binds IL2 with high affinity ($K_d = 10^{-11}$ mol/L). Both the heterodimeric and heterotrimeric receptors signal. In contrast with IL2Rα, the isolated IL15Rα has a high affinity for IL15 ($K_d = 10^{-11}$ mol/L; refs. 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 MHCs are associated elements in supramolecular receptor clusters in lipid rafts before the cytokine addition and that IL2 and IL15 compete for the use of γc (19). In 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 (19). 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-κB as well as decreasing 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 CTLs and immunoglobulin synthesis by B cells that have been stimulated with immunoglobulin M (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 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 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 expression 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-associated molecules, and exerts suppressive activity (28).

In contrast with 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 a 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 antiapoptotic 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 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. 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 cells 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, 12 kDA 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 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 the extracellular matrix binds to preform high-affinity heterotrimeric receptors at the surface of activated cells (Fig. 1; ref. 37). In contrast with 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 NF-κB 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, costimulatory 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 colleagues (45) demonstrated that IL15 *cis* presentation is required for optimal NK-cell activation in lipopolysaccharide-mediated inflammatory conditions. In addition to the classical heterotrimeric IL15 receptor and the 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, IL15 can signal through IL15Rα, JNK, and NF-κB to drive RANTES production by myeloid cells (47–49).

Disorders of the IL15/IL2 Cytokine Receptor System

The gene encoding γc is mutated in individuals with X-linked 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.

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 GM-CSF (3). The retrovirus HTLV-1–encoded tax protein expressed in adult T-cell leukemia (ATL) and the neurologic disease HAM/TSP activates two autocrine systems 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* peripheral blood mononuclear cells proliferate spontaneously.

IL2R- and IL15R-Directed Immunotherapy

The specific IL2R subunit IL2Rα has been an exceptionally valuable target for immunotherapy (9, 56–63). 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, 58). 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; Roche), the first humanized antibody that was approved by the FDA (56, 57, 59, 60). 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 noninfectious uveitis (59). Furthermore, treatment with daclizumab has resulted in an over 70% reduction in new contrast-enhancing lesions in patients with multiple sclerosis who did not respond to treatment with IFNβ (60). In addition, daclizumab was shown to provide effective therapy for selected patients with human T-cell lymphotropic virus 1–associated (HTLV-1) ATL—which has been viewed as the leukemic counterpart of Tregs (56, 63). In additional clinical trials, daclizumab armed with toxins or β-emitting radionuclides that result in selective delivery of toxins or radionuclides to cells expressing IL2Rα has provided effective therapy for selected patients with ATL and Hodgkin lymphoma (61, 62).

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 pathways (IL2/IL2R, IL15/IL15R) and one paracrine (IL9) pathway that signal through the γc and the JAK1/3–STAT3/5 pathway (54, 55). In light of this multicytokine activation in lieu of a mAb inhibition, utilizing a JAK inhibitor (e.g., ruxolitinib/tofacitinib) or more appropriately a JAK1-specific inhibitor could be of value (55).

IL2 in the Treatment of Cancer

Rosenberg (64) first utilized ultra-high doses of IL2 to treat patients with metastatic renal cell carcinoma. Such high-dose IL2 therapy resulted in a significant clinical response (around 15%); however, it also resulted in very significant toxicity. The IL2 doses used amounted to approximately 50 million units per injection every 8 hours, which 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 that induced the vascular capillary leak syndrome. IL2 (aldesleukin) 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 $CD25^+$ $Foxp3^+$ Tregs 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, 65). 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 shown disappointing results presumably due in part to the expansion of Tregs.

In addition to natural IL2, a conformational switch has been exploited to engineer an IL2 "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 (66). Furthermore, Boyman and colleagues (67) demonstrated that some IL2/anti-IL2 mAb 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. Thus, IL2 antibody complexes have been used to selectively boost the immune response and reduce tumor metastases (67–70). Furthermore, an antibody–IL2 fusion protein has been shown to overcome tumor heterogeneity by induction of a cellular immune response (69). In parallel, IL15/antibody fusion proteins for cancer immunotherapy mimicking IL15 trans-presentation at the tumor site have been generated (71).

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, 72–81). Intravenous administration of murine IL15 enhanced survival of such tumor-bearing mice (73). Furthermore, Klebanoff and colleagues (72) 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. In addition, Bessard and colleagues (82) demonstrated high antitumor activity of RLI, an IL15–IL15Rα fusion protein, in metastatic melanoma and colorectal cancer models. On the basis of the animal preclinical trials with IL15, great interest was generated among leading immunotherapeutic experts participating in the NCI Immunotherapy Agent Workshop, which ranked IL15 as the most promising unavailable immunotherapeutic agent to be brought to human therapeutic trials (83).

The safety of IL15 was evaluated in rhesus macaques by Munger and colleagues (75), Mueller and colleagues (84), Waldmann and colleagues (85), Lugli and colleagues (86), Sneller and colleagues (87), and Berger and colleagues (88). A 12-day bolus of intravenous administration of 20 µg/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 (85). Subsequently, alternative routes of administration were evaluated in rhesus macaques, including continuous intravenous infusion (CIV) and subcutaneous administration of IL15. The administration of IL15 by CIV at 20 µg/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 (87). Subcutaneous infusions at 20 µg/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 (89–92). 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 (MTD) of rhIL15 administered as a daily intravenous bolus infusion for 12 days to patients with metastatic malignant melanoma or metastatic renal cell cancer (93). The study was initially planned as a phase I dose-escalation trial starting with an initial dose of 3 μg/kg/d 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 of 1.0 and 0.3 μg/kg/d (93). Two of 4 patients given the 1.0 μg/kg/d dose had persistent grade 3 alanine aminotransferase and aspartate aminotransferase elevations that were dose limiting. All 9 patients with IL15 administered at 0.3 μg/kg/d received all 12 doses without DLT. The MTD of rhIL15 was determined to be 0.3 μg/kg/d.

There was a consistent temporal pattern of posttreatment adverse events in patients given the 3-μg/kg/day dose of IL15 with fever and rigors beginning 2.5 to 4 hours after the start of IL15 infusions and blood pressure dropping 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 CIV and subcutaneous infusions of IL15 in trials have been initiated (90, 91).

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 (94). 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 (94). 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 (95–99).

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 (73, 79). 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 with 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 (73). 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 (73).

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 Tregs (100, 101). 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; refs. 80, 102). 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 (80, 102).

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 the 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 coadministered 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 mAbs (82, 103–110). 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 antibody-dependent cell-mediated cytotoxicity action (104–109). 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, 3 of the 18 evaluable patients generated clinical responses (107). In addition, IL2 has been given in combination with rituximab for the treatment of B-cell malignancies with varying success (109). 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 (110, 111). In parallel with an IL15 conjugate, Vincent and colleagues (112) reported highly potent anti–

CD20-RLI immunocytokine targeting established human B-cell lymphoma in SCID mice. It is hoped that with the diverse approaches discussed, IL15 and IL2 will take central places in the combination treatment of cancer.

Grant Support

References

1. Sugamura K, Asao H, Kondo M, Tanaka N, Ishii N, Ohbo K, et al. The interleukin-2 receptor gamma chain: its role in the multiple cytokine receptor complexes and T cell development in XSCID. Annu Rev Immunol 1996;14:179–205.
2. Noguchi M, Nakamura Y, Russell SM, Ziegler SF, Tsang M, Cao X, et al. Interleukin-2 receptor gamma chain: a functional component of the interleukin-7 receptor. Science 1993;262:1877–80.
3. Waldmann TA. The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. Nat Rev Immunol 2006;6:595–601.
4. Hakimi J, Ha VC, Lin P, Campbell E, Gately MK, Tsudo M, et al. Humanized Mik beta 1, a humanized antibody to the IL-2 receptor beta-chain that acts synergistically with humanized anti-TAC. J Immunol 1993;151:1075–85.
5. Bamford RN, Grant AJ, Burton JD, Peters C, Kurys G, Goldman CK, et al. The interleukin (IL) 2 receptor beta chain is shared by IL-2 and a cytokine, provisionally designated IL-T, that stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. Proc Natl Acad Sci U S A 1994;91:4940–4.
6. Giri JG, Kumaki S, Ahdieh M, Friend DJ, Loomis A, Shanebeck K, et al. Identification and cloning of a novel IL-15 binding protein that is structurally related to the alpha chain of the IL-2 receptor. EMBO J 1995;14:3654–63.
7. Liao W, Lin J, Leonard WJ. IL-2 Family Cytokins: New insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. Curr Opin Immunol 2011;23:598–604.
8. Ring AM, Lin JX, Feng D, Mitra S, Rickert M, Bowman GR, et al. Mechanistic and structural insight into the functional dichotomy between IL-2 and IL-15. Nat Immunol 2012;13:1187–95.
9. Waldmann TA, Dubois S, Tagaya Y. Contrasting roles of IL-2 and IL-15 in the life and death of lymphocytes: implications for immunotherapy. Immunity 2001;14:105–10.
10. Waldmann TA. The interleukin-2 receptor. J Biol Chem 1991;266:2681–4.
11. Marks-Konczalik J, Dubois S, Losi JM, Sabzevari H, Yamada N, Feigenbaum L, et al. IL-2-induced activation-induced cell death is inhibited in IL-15 transgenic mice. Proc Natl Acad Sci U S A 2000;97:11445–50.
12. Taniguchi T, Minami Y. The IL-2/IL-2 receptor system: a current overview. Cell 1993;73:5–8.
13. Fehniger TA, Caligiuri MA. Interleukin 15: biology and relevance to human disease. Blood 2001;97:14–32.
14. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995;155:1151–64.
15. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. Nat Rev Immunol 2012;12:180–90.
16. Zhang X, Sun S, Hwang I, Tough DF, Sprent J. Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15. Immunity 1998;8:591–9.
17. Bamford RN, Battiata AP, Burton JD, Sharma H, Waldmann TA. Interleukin (IL) 15/IL-T production by the adult T-cell leukemia cell line HuT-102 is associated with a human T-cell lymphotrophic virus type I R region/IL-15 fusion message that lacks many upstream AUGs that normally attenuate IL-15 mRNA translation. Proc Natl Acad Sci U S A 1996;93:2897–902.
18. Bamford RN, DeFilippis AP, Azimi N, Kurys G, Waldmann TA. The 5′ untranslated region, signal peptide and the coding sequence of the carboxyl terminus of IL-15 participate in its multifaceted translational control. J Immunol 1998;160:4418–26.
19. Damjanovich S, Bene L, Matkó J, Alileche A, Goldman CK, Sharrow S, et al. Preassembly of interleukin 2 (IL-2) receptor subunits on resting Kit 225 K6 T cells and their modulation by IL-2, IL-7 and IL15: a fluorescence resonance energy transfer study. Proc Natl Acad Sci U S A 1997;94:13134–9.
20. Mishra A, Sullivan L, Caligiuri MA. Molecular pathways: interleukin-15 signaling in health and in cancer. Clin Cancer Res 2014;20:2044–50.
21. Wang X, Ricket M, Garcia KC. Structure of the quaternary complex of interleukin-2 with its alpha, beta and gamma-c receptors. Science 2005;310:1159–63.
22. Steel JC, Waldmann TA, Morris JC. Interleukin-15 biology and its therapeutic implications in cancer. Trends Pharmacol Sci 2012;33:35–41.
23. Johnston JA, Bacon CM, Finbloom DS, Rees RC, Kaplan D, Shibuya K, et al. Tyrosine phosphorylation and activation of STAT5, STAT3, and Janus kinases by interleukins 2 and 15. Proc Natl Acad Sci U S A 1995;92:8705–9.
24. Marcais A, Cherfils-Vicini J, Viant C, Degouve S, Viel S, Fenis A, et al. The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. Nat Immunol 2014;15:749–57.
25. Cantrell D. Signaling in lymphocyte activation. Cold Spring Harb Perspect Biol. 2014 doi 10.1101/cshperspect.col8788.
26. Alexander WS, Hilton DJ. The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immune system. Annu Rev Immunol 2004;22:503–29.
27. Carson WE, Fehniger TA, Haldar S, Eckhert K, Lindemann MJ, Lai CF, et al. A potential role for interleukin-15 in the regulation of human natural killer cell survival. J Clin Invest 1997;99:937–43.
28. Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 2005;6:345–52.
29. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin-2 in Foxp3-expressing regulatory T cells. Nat Immunol 2005;6:1142–51.
30. Maloy KJ, Powrie F. Fueling regulation: IL-2 keeps CD4+ Treg cells fit. Nat Immunol 2005;6:1071–2.
31. Ono M, Yaguchi H, Ohkura N, Kitabayashi I, Nagamura Y, Nomura T, et al. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. Nature 2007;446:685–9.
32. Lenardo MJ. Fas and the art of lymphocyte maintenance. J Exp Med 1996;183:721–4.
33. Sadlack B, Kühn R, Schorle H, Rajewsky K, Müller W, Horak I. Development and proliferation of lymphocytes in mice deficient for both interleukins-2 and-4. Eur J Immunol 1994;24:281–4.
34. Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M, et at. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. J Exp Med 2000;191:771–800.

35. Waldmann TA, Dubois S, Muller J, Goldman C, Damjanovich S. Biophysical aspects of transmembrane signaling. Damjanovich S, editor. Heidelberg, Germany: Springer; 2005. p. 97–121

36. Dubois S, Shou W, Haneline LS, Fleischer S, Waldmann TA, Müller JR. Distinct pathways involving the FK506-binding proteins 12 and 12.6 underlie IL-2-versus IL-15 mediated proliferation of cells. Proc Natl Acad Sci U S A 2003;100:14169–74.

37. Dubois S, Mariner J, Waldmann TA, Tagaya Y. IL-15Ralpha recycles and presents IL-15 In trans to neighboring cells. Immunity 2002; 17:537–47.

38. Schluns KS, Klonowski KD, Lefrancois L. Transregulation of memory CD8 T-cell proliferation by IL-15Ralpha+ bone marrow-derived cells. Blood 2004;103:988–94.

39. Mortier E, Woo T, Advincula R, Gozalo S, Ma A. IL-15R alpha chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation. J Exp Med 2008;205:1213–25.

40. Chirifu M, Hayashi C, Nakamura T, Toma S, Shuto T, Kai H, et al. Crystal structure of the IL-15-IL-15R alpha complex, a cytokine-receptor unit presented in trans. Nat Immunol 2007;8:1001–7.

41. Olsen SK, Ota N, Kishishita S, Kukimoto-Nino M, Murayama K, Uchiyama H, et al. Crystal structure of the interleukin-15. interleukin-15 receptor alpha complex: insights into trans and cis presentation. J Biol Chem 2007;282:37191–204.

42. Wuest SC, Edwan JH, Martin JF, Han S, Perry JS, Cartagena CM, et al. A role for interleukin-2 trans-presentation in dendritic cell-mediated T cell activation in humans, as revealed by daclizumab therapy. Nat Med 2011;17:604–9.

43. Rowley J, Monie A, Hung CF, Wu TC. Expression of IL-15RA or an IL-15/IL-15RA fusion on CD8+ T cells modifies adoptively transferred T-cell function in cis. Eur J Immunol 2009;39:491–506.

44. Waldmann TA, Conlon KC, Stewart DM, Worthy TA, Janik JE, Fleisher TA, et al. Phase 1 trial of IL-15 trans presentation blockade using humanized Mik-Beta-1 mAb in patients with T-cell large granular lymphocytic leukemia. Blood 2013;121:476–84.

45. Zanoni I, Spreafico R, Bodio C, Di Gioia M, Cigni C, Broggi A, et al. IL-15 cis presentation is required for optimal NK cell activation in lipopolysaccharide-mediated inflammatory conditions. Cell Rep 2013; 4:1235–49.

46. Tagaya Y, Burton JD, Miyamoto Y, Waldmann TA. Identification of a novel receptor/signal transduction pathway for IL-15/T in mast cells. EMBO J 1996;15:4928–39.

47. Chenoweth MJ, Mian MF, Barra NG, Alain T, Sonenberg N, Bramson J, et al. IL-15 can signal via IL-15R alpha, JNK, and NF-kB to drive RANTES production by myeloid cells. J Immunol 2012;188:4149–57.

48. Pereno R, Giron-Michel J, Gaggero A, Cazes E, Meazza R, Monetti M, et al. IL-15/IL-15Ralpha intracellular trafficking in human melanoma cells and signal transduction through the IL-15Ralpha. Oncogene 2000; 19:5153–62.

49. Bulfone-Paus S, Bulanova E, Budagian V, Paus R. The interleukin-15/interleukin-15 receptor system as a model for juxtacrine and reverse signaling. Bioessays 2006;28:362–77.

50. Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS, et al. Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. Cell 1993; 73:147–57.

51. Macchi P, Villa A, Giliani S, Sacco MG, Frattini A, Porta F, et al. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). Nature 1995;377:65–8.

52. Russell SM, Tayebi N, Nakajima H, Riedy MC, Roberts JL, Aman MJ, et al. Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. Science 1995;270:797–800.

53. Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity 2007;26:371–81.

54. Chen J, Petrus M, Bryant BR, Phuc Nguyen V, Stamer M, Goldman CK, et al. Induction of the IL-9 gene by HTLV-I Tax stimulates the spontaneous proliferation of primary adult T-cell leukemia cells by a paracrine mechanism. Blood 2008;111:5163–72.

55. Ju W, Zhang M, Jiang JK, Thomas CJ, Oh U, Bryant BR, et al. CP-690,550, a therapeutic agent, inhibits cytokine-mediated Jak3 activation and prolif-

56. eration of T cells from patients with ATL and HAM/TSP. Blood 2011;117:1938–46.

56. Waldmann TA. Anti-Tac (daclizumab, Zenapax) in the treatment of leukemia, autoimmune diseases, and in the prevention of allograft rejection: A 25-year-personal odyssey. J Clin Immunol 2007;27: 1–18.

57. Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, Halloran P, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. N Engl J Med 1998;338:161–5.

58. Kuhn DJ, Dou QP. The role of interleukin-2 receptor alpha in cancer. Front Biosci 2005;10:1462–74

59. Nussenblatt RB, Fortin E, Schiffman R, Rizzo L, Smith J, Van Veldhuisen P, et al. Treatment of noninfectious intermediate and posterior uveitis with the humanized anti-Tac mAb: a Phase I/II clinical trial. Proc Natl Acad Sci U S A 1999;96:7462–6.

60. Bielekova B, Richert N, Howard T, Blevins G, Markovic-Plese S, McCartin J, et al. Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon beta. Proc Natl Acad Sci U S A 2004;101:8705–8.

61. Waldmann TA, White JD, Carrasquillo JA. Radioimmunotherapy of interleukin-2R alpha-expressing adult T-cell leukemia with Yttrium-90-labeled anti-Tac. Blood 1995;86:4063–75.

62. Kreitman RJ, Wilson WH, White JD, Stetler-Stevenson M, Jaffe ES, Giardina S, et al. Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. J Clin Oncol 2000;18:1622–36.

63. Berkowitz JL, Janik JE, Stewart DM, Jaffe ES, Stetler-Stevenson M, Shih JH, et al. Safety, efficacy, and pharmacokinetics/pharmacodynamics of daclizumab (anti-CD25) in patients with adult T-cell leukemia/lymphoma. Clin Immunol 2014;155:176–87.

64. Rosenberg SA. Interleukin-2 and the development of immunotherapy for the treatment of patients with cancer. Cancer J Sci Am 2000;6:Suppl 1: S2–S7.

65. Smith KA. A compendium of cytokines and other mediators of host defense, pages 113–126. Volume 1, Ligands. Edited by Oppenheim J.J. and Feldmann M. Waltham (MA): Academic Press; 2001.

66. Levin AM, Bates DL, Ring AM, Krieg C, Lin JT, Su L, et al. Exploiting a natural conformational switch to engineer an interleukin-2 `superkine'. Nature 2012;484:529–33.

67. Boyman O, Kovar M, Rubinstein MP, Surh CD, Sprent J. Selective stimulation of T cell subsets with antibody-cytokine immune complexes. Science 2006;311:1924–7.

68. Jin GH, Hirano T, Murakami M. Combination treatment with IL-2 and anti-IL-2 mAbs reduces tumor metastasis via NK cell activation. Int Immunol 2008;20:783–9.

69. Becker JC, Varki N, Gillies SD, Furukawa K, Reisfeld RA. An antibody-interleukin 2 fusion protein overcomes tumor heterogeneity by induction of a cellular immune response. Proc Natl Acad Sci U S A 1996;93:7826–31.

70. Reisfeld RA, Gillies SD. Antibody-interleukin 2 fusion proteins: a new approach to cancer therapy. J Clin Lab Anal 1996;10:160–6.

71. Kermer V, Baum V, Hornig N, Kontermann RE, Müller D. An antibody fusion protein for cancer immunotherapy mimicking IL-15 trans-presentation at the tumor site. Mol Cancer Ther 2012; 11:1279–88.

72. Klebanoff CA, Finkelstein SE, Surman DR, Lichtman MK, Gattinoni L, Theoret MR, et al. IL-15 enhances the *in vivo* antitumor activity of tumor-reactive CD8+ T cells. Proc Natl Acad Sci U S A 2004;101:1969–74.

73. Zhang M, Ju W, Yao Z, Yu P, Wei BR, Simpson RM, et al. Augmented IL-15Ralpha expression by CD40 activation is critical in synergistic CD8 T cell-mediated antitumor activity of anti-CD40 antibody with IL-15 in TRAMP-C2 tumors in mice. J Immunol 2012;188:6156–64.

74. Kobayashi H, Dubois S, Sato N, Sabzevari H, Sakai Y, Waldmann TA, et al. Role of trans-cellular IL-15 presentation in the activation of NK cell-mediated killing, which leads to enhanced tumor immunosurveillance. Blood 2005;105:721–7.

75. Munger W, DeJoy SQ, Jeyaseelan R Sr, Torley LW, Grabstein KH, Eisenmann J, et al. Studies evaluating the antitumor activity and toxicity of interleukin-15, a new T cell growth factor: comparison with interleukin-2. Cell Immunol 1995;165:289–93.

76. Evans R, Fuller JA, Christianson G, Krupke DM, Troutt AB. IL-15 mediates anti-tumor effects after cyclophosphamide injection of tumor-bearing mice and enhances adoptive immunotherapy: the potential role of NK cell subpopulations. Cell Immunol 1997;179:66–73.

77. Zeng R, Spolski R, Finkelstein SE, Oh S, Kovanen PE, Hinrichs CS, et al. Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. J Exp Med 2005;201:139–48.

78. Dubois S, Patel HJ, Zhang M, Waldmann TA, Müller JR. Preassociation of IL-15 with IL-15R alpha-IgG1-Fc enhances its activity on proliferation of NK and CD8+/CD44 high T cells and its antitumor action. J Immunol 2008;180:2099–106.

79. Zhang M, Yao Z, Dubois S, Ju W, Müller JR, Waldmann TA. Interleukin-15 combined with an anti-CD40 antibody provides enhanced therapeutic efficacy for murine models of colon cancer. Proc Natl Acad Sci U S A 2009;106:7513–8.

80. Yu P, Steel JC, Zhang M, Morris JC, Waitz R, Fasso M, et al. Simultaneous inhibition of two regulatory T-cell subsets enhanced Interleukin-15 in a prostate tumor model. Proc Natl Acad Sci U S A 2012; 109:6187–92.

81. Roychowdhury S, May KF Jr, Tzou KS, Lin T, Bhatt D, Freud AG, et al. Failed adoptive immunotherapy with tumor-specific T cells: reversal with low-dose interleukin15 but not low-dose interleukin 2. Cancer Res 2004;64:8062–7.

82. Bessard A, Solé V, Bouchaud G, Quéméner A, Jacques Y. High antitumor activity of RLI, an interleukin-15 (IL-15)-IL-15 receptor alpha fusion protein, in metastatic melanoma and colorectal cancer. Mol Cancer Ther 2009;8:2736–45.

83. NCI Immunotherapy Agent Worskshop Executive Summary. July 12, 2007. p. 1–4; no authors.

84. Mueller YM, Petrovas C, Bojczuk PM, Dimitriou ID, Beer B, Silvera P, al. Interleukin-15 increases effector memory CD8+ T cells and NK cells in simian immunodeficiency virus-infected macaques. J Virol 2005;79: 4877–85.

85. Waldmann TA, Lugli E, Roederer M, Perera LP, Smedley JV, Macallister RP, et al. Safety (toxicity), pharmacokinetics, immunogenicity, and impact on elements of the normal immune system of recombinant human IL-15 in rhesus macaques. Blood 2011;117:4787–95.

86. Lugli E, Goldman CK, Perera LP, Smedley J, Pung R, Yovandich JL, et al. Transient and persistent effects of IL-15 on lymphocyte homeostasis in nonhuman primates. Blood 2010;116:3238–48.

87. Sneller MC, Kopp WC, Engelke KJ, Yovandich JL, Creekmore SP, Waldmann TA, et al. IL-15 administered by continuous intravenous infusion to rhesus macaques induces massive expansion of the CD8+ T effector memory population in peripheral blood. Blood 2011; 118:6845–8.

88. Berger C, Berger M, Hackman RC, Gough M, Elliott C, Jensen MC, et al. Safety and immunological effects of IL-15 administration in nonhuman primates. Blood 2009;114:2417–26.

89. A phase I study of intravenous recombinant human IL-15 in adults with refractory metastatic malignant melanoma and metastatic renal cell cancer. Available from: https://clinicaltrials.gov/ct2/results?term=NCT01021059+&Search=Search

90. Recombinant interleukin-15 in treating patients with advanced melanoma, kidney cancer, non-small cell lung cancer, or squamous cell head and neck cancer. Available from: https://clinicaltrials.gov/ct2/results?term=NCT01727076+&Search=Search

91. Continuous infusion of rhIL-15 for adults with advanced cancer. Available from: https://clinicaltrials.gov/ct2/results?term=NCT01572493+&Search=Search

92. Haploidentical donor natural killer cell infusion with IL-15 in acute myelogenous leukemia (AML). Available from: https://clinicaltrials.gov/ct2results?term=NCT01385423+&Search=Search

93. Conlon KC, Lugli E, Welles HC, Rosenberg SA, et al. al. Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer. J Clin Oncol 2014;33:74–82. [Epub ahead of print].

94. Chen J, Petrus M, Bamford R, Shih JH, Morris JC, Janik JE, et al. Increased serum soluble IL-15Rα levels in T-cell large granular lymphocyte leukemia. Blood 2012;119:137–43.

95. Tinhofer I, Marschitz I, Henn T, Egle A, Greil R. Expression of functional interleukin-15 receptor and autocrine production of interleukin-15 as mechanisms of tumor propagation in multiple myeloma. Blood 2000;95:610–8.

96. Chertova E, Bergamaschi C, Chertov O, Sowder R, Bear J, Roser JD, et al. Characterization and favorable in vivo properties of heterodimeric soluble IL-15-IL-15Rα cytokine compared to IL-15 monomer. J Biol Chem 2013;288:18093–103.

97. Altor launches clinical trial of IL-15 superagonist protein complex against metastatic melanoma. Cellular Technology LTd, 2013. Available from: http://clinicaltrialspharmaceutical-business-review.com/news/alto-launches-clinicaltrial

98. Stoklasek TA, Schluns KS, Lefrancois L. Combined IL-15/IL-15Ralpha immunotherapy maximizes IL-15 activity in vivo. J Immunol 2006;177: 6072–80.

99. Rubinstein MP, Kovar M, Purton JF, Cho JH, Boyman O, Surh CD, et al. Converting IL-15 to a superagonist by binding to soluble IL-15R{alpha}. Proc Natl Acad Sci U S A 2006;103:9166–71.

100. Lee YH, Ishida Y, Rifa'i M, Shi Z, Isobe K, Suzuki H. Essential role of CD8+CD122+ regulatory T cells in the recovery from experimental autoimmune encephalomyelitis. J Immunol 2008;180:825–32.

101. Endharti AT, Rifa'l M, Shi Z, Fukuoka Y, Nakahara Y, Kawamoto Y, et al. Cutting edge: CD8+CD122+ regulatory T cells produce IL-10 to suppress IFN-gamma production and proliferation of CD8+ T cells. J Immunol 2005;175:7093–7.

102. Yu P, Steel JC, Zhang M, Morris JC, Waldmann TA. Simultaneous blockade of multiple immune system inhibitory checkpoints enhances antitumor activity mediated by interleukin-15 in a murine metastatic colon carcinoma model. Clin Can Res 2010;16: 6019–28.

103. Oh S, Berzofsky JA, Burke DS, Waldmann TA, Perera LP. Coadministration of HIV vaccine vectors with vaccinia viruses expressing IL-15 but not IL-2 induces long-lasting cellular immunity. Proc Natl Acad Sci U S A 2003;100:3392–7.

104. Roberti MP, Barrio MM, Bravo Al, Rocca YS, Arriaga JM, Bianchini M, et al. IL-15 and IL-2 increase Cetuximab-mediated cellular cytotoxicity against triple negative breast cancer cell lines expressing EGFR. Breast Cancer Res Treat 2011;130:465–75.

105. Moga E, Alvarez E, Cantó E, Vidal S, Rodriguez-Sánchez JL, Sierra J, et al. NK cells stimulated with IL-15 or CpG ODN enhance rituximab-dependent cellular cytotoxicity against B-cell lymphoma. Exp Hematol 2008;36:69–77.

106. Sondel PM, Hank JA. Combination therapy with interleukin-2 and antitumor monoclonal antibodies. Cancer J Sci Am 1997;3:S121–7.

107. Soiffer RJ, Chapman PB, Murray C, Williams L, Unger P, Collins H, et al. Administration of R24 monoclonal antibody and low-dose interleukin 2 for malignant melanoma. Clin Cancer Res 1997;3: 17–24.

108. Shak S. Overview of the trastuzumab (Herceptin) anti-HER2 monoclonal antibody clinical program in HER2-overexpressing metastatic breast cancer. Herceptin Multinational Investigator Study Group. Semin Oncol 1999;26:71–7.

109. Keilholz U, Szélenyi H, Siehl J, Foss HD, Knauf W, Thiel E. Rapid regression of chemotherapy refractory lymphocyte predominant Hodgkin's disease after administration of rituximab (anti-CD20 mono-clonal antibody) and interleukin-2. Leuk Lymphoma 1999;35: 641–2.

110. Penichet ML, Morrison SL. Antibody-cytokine fusion proteins for the therapy of cancer. J Immunol Methods 2001;248:91–101.

111. Lode HN, Reisfeld RA. Targeted cytokines for cancer immunotherapy. Immunol Res 2000;21:279–88.

112. Vincent M, Teppaz G, Lajoie L, Solé V, Bessard A, Maillasson M, et al. Highly potent anti-CD20-RLI immunocytokine targeting established human B lymphoma in SCID mouse. MAbs 2014;6:1026–37.

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The Shared and Contrasting Roles of IL2 and IL15 in the Life and Death of Normal and Neoplastic Lymphocytes: Implications for Cancer Therapy

Thomas A. Waldmann

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