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It was the early 1990s, and there was an unsolved mystery in innate immunity: what cytokine was responsible for supporting the development and survival of NK cells? We understood that the common γ-chain ($\gamma_c$) cytokine receptor was critical because patients with SCID due to *IL-2RG* mutations had markedly reduced NK cell function (1, 2). The prevailing thought in the field was that IL-2, which signals via a high-affinity heterotrimeric receptor, IL-2Rαβγ, was the most likely candidate. After all, IL-2 resulted in potent NK cell activation, proliferation, and survival in vitro. However, all of the facts did not add up; IL-2 is produced mainly by activated T cells, which are spatially and temporally disconnected from normal NK cell development and homoeostasis. Moreover, mice that had disrupted genes for IL-2 (3) or the IL-2Rα (4) did not have a defect in NK cells. What clandestine cytokine used the IL-2R to support NK cells?

The mystery was unraveled in landmark reports by two independent groups that employed a simple strategy to search for alternative IL-2R ligands: examining the composition of cell line supernatants capable of supporting IL-2R–dependent growth signals in the presence of anti-IL-2–neutralizing Abs (5, 6). Scientists at Immunex Corporation used chromatography to purify a 14-kDa IL-2–functional mimic from the simian CV-1/EBNA cell line supernatant, sequenced NH2-terminal residues, and then used degenerate oligonucleotide primers to clone a full-length cDNA. This approach ultimately revealed a novel cytokine designated IL-15 (this article is available at https://science.sciencemag.org/content/264/5161/965) (5). IL-15 used the IL-2Rβ and $\gamma_c$ (7) but did not require the IL-2Rα, and IL-15 promoted NK cell activation and proliferation (8), matching the profile of the mystery cytokine. Concurrently, scientists at the National Institutes of Health, led by Thomas Waldmann, identified functional IL-2–mimicry activity in HTLV-1–transformed HuT-102 cell line supernatant and isolated a cytokine that was initially designated IL-T (this article is available at https://www.pnas.org/content/pnas/91/11/4935.full.pdf) (6). IL-T stimulated T cell proliferation and generated lymphokine-activated killer cells, required the IL-2Rβ and $\gamma_c$ but not IL-2Rα, and thus also fit the cytokine puzzle. Reconciling IL-T and IL-15, mature IL-T was found to be identical to IL-15. Indeed, the gene encoding IL-T/IL-15 within HuT-102 was fused to the HTLV-1 LTR that had inserted into the 5′UTR of IL-T. This resulted in enhanced transcription, translation, and secretion of IL-T/IL-15 (9). IL-15 mRNA was found in a wide variety of tissues with an expression profile distinct from IL-2, also allowing for greater cytokine access to NK cells and nonclassical T cells. Further analysis revealed disparate IL-2 and IL-15 primary sequences, but protein modeling indicated that IL-15 folded into a 4-α helix bundle cytokine, similar to IL-2. Mystery solved, and a new cytokine supporting NK cell homeostasis was discovered!

Definitive evidence of IL-15's nonredundant role in NK cell development and survival followed from IL-15 gene loss-of-function studies in mice (10). The importance of IL-15 was identified not only for NK cells but also several nonclassical T cell lineages and memory CD8 T cells. Gain-of-function studies using IL-15 transgenic mice complemented these findings, demonstrating an expansion of NK cells and memory-phenotype CD8 T cells but cautioning that chronic, unrestrained IL-15 can contribute to the development of T/NK leukemias (11). Thus, IL-15 is the primary cytokine responsible for IL-2/15Rβ$\gamma_c$ signals that promote and sustain NK cell development and survival.

Although the similarities between IL-2 and IL-15, including shared signaling receptor subunits, were instrumental in the discovery of IL-15, these same parallels influenced our initial understanding of IL-15R biology. After IL-15's discovery, mice with genetic deletions of the $\gamma_c$ (12) and IL-2/15Rβ (13) were shown to lack NK cells, solidifying the importance of IL-2/15Rβ$\gamma_c$ signaling in NK cell development. IL-2 interacts with several forms of the IL-2R with differing affinities; it has intermediate (nanomolar) affinity for the heterodimeric IL-2Rβγ and high (picomolar) affinity for the heterotrimeric IL-2αβγ, which includes a "private" α receptor subunit. However, IL-2Rα was not involved in IL-15 signaling, and a new search was on for additional IL-15R components responsible for high-affinity IL-15 binding. Almost immediately, IL-15Rα was identified and cloned based on structural homology to the IL-2Rα (14, 15). However, unlike IL-2Rα, which has low affinity for IL-2 on its own, IL-15Rα bound IL-15 with high affinity. This receptor biology leads to a situation in which limiting amounts of IL-2 are preferentially sensed by immune cells expressing IL-2Rα in the context of the IL-2Rαβγ. Indeed, ultra-low dose IL-2 therapy in humans

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Abbreviation used in this article: $\gamma_c$, common γ-chain.



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expanded subsets of NK cells that expressed the IL-2Rαβγ (16) and was later recognized to also expand IL-2Rα$^+$ regulatory T cells (17). In contrast, IL-15Rα on its own has high affinity for IL-15, which provides the opportunity to operate in *cis* when interacting with IL-15Rβγ$_c$ on the same cell or in *trans* with IL-15Rα presenting IL-15 to IL-15Rβγ$_c$ on a neighboring cell. A new mystery: which was the primary mode of IL-15 interaction with its signaling receptor components? In elegant studies using IL-15$^{-/-}$ and IL-15Rα$^{-/-}$ mice and bone marrow chimera approaches, Averil Ma's group provided clear in vivo evidence that IL-15 *trans*-presentation by IL-15Rα to NK cells expressing the IL-2/15Rβγ$_c$ was the major mode supporting NK cell development and homeostasis (18–20). Thus, the initial similarity in biology between IL-2R and IL-15R has been revised, and we now know that these cytokines mediate fundamentally different functions in vivo, in part because of this distinct receptor biology.

Twenty-five years after the discovery of IL-15, the importance of solving these mysteries is more evident than ever with the clinical translation of IL-15 to treat human disease. Initially, the biology of IL-15 was thought to be similar to FDA-approved human rIL-2 and was therefore not pursued as a therapeutic drug. With more appreciation of the distinct biology between IL-2 and IL-15, IL-15R agonists now represent an emerging and exciting immunotherapy strategy to promote NK and CD8 T cell responses in the context of infection and cancer. In 2015, the first clinical trial demonstrated NK and CD8 T cell expansion and activation in response to human rIL-15 (21). Armed with a deeper understanding of IL-15R biology, new therapeutic agents have been developed to improve in vivo pharmacokinetics and mimic physiologic IL-15:IL-15Rα *trans*-presentation. These therapeutics have been shown to be safe and promote expansion and activation of NK and CD8 T cells without expanding regulatory T cells (22). Thus, in human patients, IL-15 has now been shown to possess distinct immunomodulatory properties compared with IL-2. These IL-15R agonists hold particular promise as combination agents with other forms of immunotherapy, including cellular therapies, tumor-targeting therapeutic Abs, inhibitory checkpoint blockade, and vaccines. Thus, although IL-15's discovery solved an immunologic mystery in 1994, its recent application as a cancer immunotherapy appears promising, and only time will tell us the story of IL-15's full impact on human health and disease.

## Disclosures

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