Insights into the anticancer mechanisms of interleukin-15 from engineered cytokine therapies

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Innovative approaches in the field of cytokine engineering are revolutionizing the cancer therapeutic landscape. The IL-15 cytokine is particularly enticing as a cancer immunotherapy due to its natural propensity for stimulating the proliferation and activation of NK and CD8$^+$ T cells. In a recent IL-15 engineering approach, the cytokine was conjugated to polyethylene glycol, and the resulting molecule (NKTR-255) exhibited potent antitumor activities. In this issue of the *JCI*, Robinson et al. mechanistically explored NKTR-255 and compared its immune profile to that of the unconjugated IL-15 cytokine. The authors found that NKTR-255 employs distinct activities on NK compared with CD8$^+$ T cells. NKTR-255 signaling also showed less dependence on the expression of the IL-15 receptor-α (IL-15Rα) chain compared with unconjugated IL-15. Collectively, these findings will advance IL-15–based clinical therapies and, more generally, benefit the field of cancer immunotherapy.

IL-15 at the front line of cancer therapies

The ability to target and stimulate specific immune cell subsets with engineered molecules has brought immunotherapies to the forefront of cancer treatments. Although the field of cancer-directed immunotherapies is vast, many strategies have focused on using protein drugs derived from natural cytokines to promote the proliferation and activation of cytotoxic lymphocytes. Early cytokine-based therapies used to treat cancer, such as IFN-α and IL-2, showed moderate clinical success, but these molecules are limited by systemic toxicities and off-target effects that counteract antitumor activity (1). Developing protein therapeutics that can potently activate specific immune cell subsets with limited pleiotropy and toxicity is currently a major objective in the field of cancer drug development.

The IL-15 cytokine has emerged as a prime therapeutic candidate due to its promotion of antitumor immune responses with limited off-target effects. IL-15 has specific activity toward stimulating the proliferation and enhancing the cytolytic activity of two lymphocyte lineages, NK cells and CD8$^+$ T cells (2). IL-15 engages three receptor chains: the nonsignaling IL-15 receptor-α (IL-15Rα) subunit and the signaling chains IL-2Rβ and common γ (γ_c). Several modes of action for endogenous IL-15 have been discovered: neighboring cells, typically monocytes and DCs, can present IL-15 in complex with IL-15Rα to initiate signaling in trans, or IL-15 can be shuttled to the cell surface in complex with IL-15Rα and be delivered to the IL-2Rβ and γ_c receptor chains to initiate signaling in cis (Figure 1, A and B) (3). Studies that administered soluble IL-15 in mouse models of cancer have demonstrated its ability to stimulate NK and CD8$^+$ T cells and have established its effectiveness in slowing tumor growth, extending survival, and even preventing tumor recurrence (4). Unfortunately, early attempts to use soluble IL-15 as an anticancer agent did not translate well to clinical trials, largely due to the poor pharmacokinetic properties and weak potency of the cytokine (2). The weak activity of IL-15 has been attributed to the fact that it does not act independently as a soluble molecule, but rather is constitutively coupled to IL-15Rα.

To compensate for the shortcomings of IL-15, newer so-called IL-15 "superagonists," which are fusion proteins that couple IL-15 to a soluble form of IL-15Rα and an antibody Fc domain and that improve potency and extend serum half-life, have been developed. Many of these IL-15 superagonists show enhanced activity and therapeutic efficacy compared with soluble IL-15 in preclinical tumor models and are currently undergoing phase I clinical trials (2). Although human trials of these molecules are pushing forward, there are still obstacles ahead. For example, emerging evidence suggests that the bias of current IL-15 superagonists toward NK cells could hinder therapeutic efficacy due to NK cell exhaustion (5–7). Pitfalls such as this highlight the urgent and unmet need to elucidate the mechanistic activities of IL-15–based therapies and leverage these insights to design improved IL-15 agonists. A recent approach implemented an alternative strategy in lieu of receptor fusion in which the IL-15 cytokine was chemically conjugated to PEG to recapitulate the function of IL-15 with a prolonged serum half-life (8). The resulting molecule, dubbed NKTR-255, showed receptor affinity and signaling properties similar to those of unconjugated IL-15, but exhibited a 90-fold longer serum half-life. Importantly, NKTR-255 demonstrated potent antitumor activity in a mouse model of B cell lymphoma, making it a promising candidate in the IL-15 therapeutic space.

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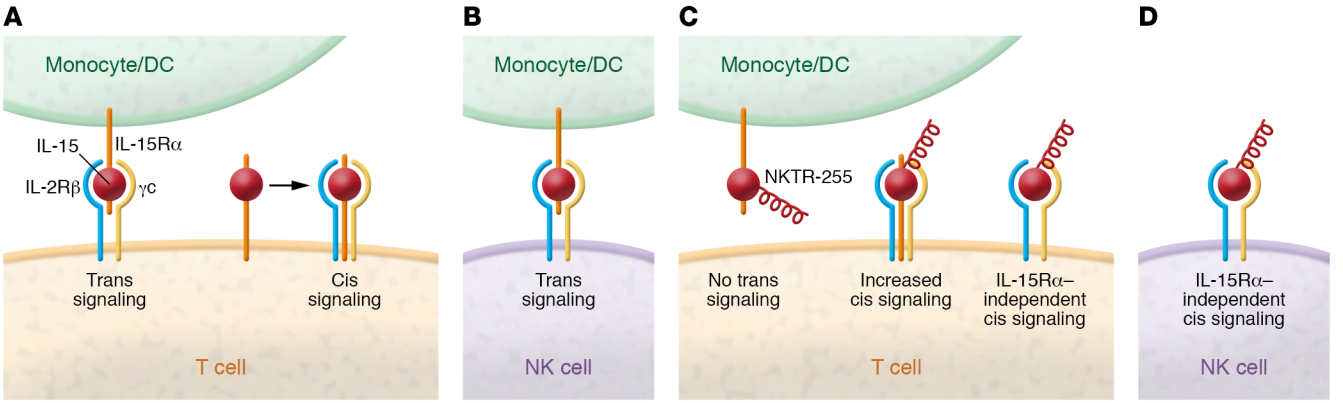

Figure 1. Molecular mechanisms of IL-15 versus NKTR-255 signaling on immune cells. (**A**) Unconjugated IL-15 signals through a complex of the receptor chains IL-15Rα, IL-2Rβ, and γ_c. On T cells, IL-15 signals either in trans, wherein precomplexed IL-15:IL-15Rα is presented by a neighboring cell to the stimulated cell, or in cis, wherein the entire complex assembles on the stimulated cell. (**B**) IL-15 stimulation of NK cells is primarily mediated through trans signaling. (**C**) NKTR-255 signals on CD8$^+$ T cells in cis and, with reduced potency, through an IL-15Rα–independent mechanism. (**D**) NK cells are solely stimulated by an IL-15Rα–independent mechanism.

Defining the immunomodulatory properties of an IL-15 therapeutic

Given the preliminary success of NKTR-255 in preclinical models, Robinson et al. (9) sought to dissect the mechanisms underlying the potent immunostimulatory functions of this PEGylated IL-15 molecule. In particular, the researchers sought to determine whether NKTR-255 activities were mediated via trans signaling from neighboring cells or via cis signaling on IL-15–responsive cells. The authors established that NKTR-255 induced proliferation of both CD8$^+$ T and NK cells, but not CD4$^+$ T cells. Moreover, the investigators showed that NKTR-255 treatment also expanded memory CD8$^+$ T cell subsets, particularly central memory cells, and induced NK cell maturation.

Trans presentation of IL-15 is likely the predominant mechanism that promotes lymphocyte development and homeostasis (10), and indeed the authors demonstrated that NKTR-255 could be presented from cell surface–localized IL-15Rα in animal models. Unexpectedly, knockout of host IL-15Rα expression failed to impair NKTR-255–mediated proliferation of either naive or memory CD8$^+$ T cells, suggesting that trans presentation was not in fact an important mechanism for NKTR-255 signaling on T cells (Figure 1C). This finding contrasted starkly with analogous studies involving the unconjugated IL-15 cytokine, which showed that IL-15–induced memory T cell expansion occurred only in the presence, but not the absence, of host IL-15Rα expression (Figure 1A). These differences in trans signaling utilization between NKTR-255 and IL-15 are likely due to interference with trans presentation that arises from PEG conjugation. Yet more mechanistic distinctions were observed between NKTR-255 and human IL-15 in terms of cis presentation. Whereas NKTR-255 relied to some extent on cis presentation in both naive and memory CD8$^+$ T cells (Figure 1C), no such dependence was observed for IL-15 (Figure 1A). Interestingly, cis presentation was not found to be relevant for NKTR-255–induced expansion of NK cells, indicating that NKTR-255 activates the IL-2Rβ/γ_c heterodimeric signaling complex independently of IL-15Rα on this immune cell lineage (Figure 1D). Taken together, these findings establish that the biological activity of NKTR-255 fundamentally differs from that of the unconjugated IL-15 cytokine and that these differences have important implications for immune responses to the two molecules.

To better understand the interplay between soluble IL-15Rα and surface-bound receptor in the context of NKTR-255 treatment, the investigators employed a transgenic mouse model in which the murine IL-15Rα subunit was expressed only on intestinal epithelial cells, but was absent from the rest of the body. Interestingly, NKTR-255–treated mice with elevated levels of soluble IL-15R showed blunted naive and memory CD8$^+$ T cells as well as NK cell proliferation responses compared with counterparts with low levels of soluble IL-15Rα, suggesting competition between soluble IL-15Rα and surface-bound receptor. Moreover, the presence of surface IL-15Rα did not noticeably affect the T cell response to NKTR-255. Subsequent studies revealed that, although high endogenous levels of soluble mouse IL-15Rα were found to inhibit NKTR-255 activity, the T and NK cell expansion activities of the PEGylated IL-15 were actually potentiated by delivery in complex with a mouse IL-15Rα Fc fusion protein. In contrast, delivery of NKTR-255 in complex with a human IL-15Rα Fc fusion protein antagonized the proliferative functions of NKTR-255 on naive and memory CD8$^+$ T cells as well as NK cells. The paradoxical effects of mouse and human IL-15Rα fusion proteins on the immunostimulatory properties of NKTR-255 parallel their activities on the unconjugated IL-15 cytokine (11, 12), indicating that NKTR-255 and unconjugated IL-15 are similarly engaged by soluble IL-15Rα and that their activities are highly sensitive to the presence of the soluble receptor.

Future work and perspectives

Several approaches have been adopted to enhance the immunostimulatory activities of the IL-15 cytokine. Most efforts have focused on either fusing or complexing IL-15 with the IL-15Rα receptor and an antibody Fc domain to create potent and durable immune agonists that act independently of soluble or surface-bound

IL-15Rα (13). Alternatively, the IL-15 cytokine itself has been engineered to increase its affinity 10-fold for the IL-2Rβ/γ_c heterodimeric receptor complex (14). This cytokine mutation strategy has in turn been combined with an IL-15Rα-Fc domain fusion protein to further exaggerate the agonistic activities and extend the half-life of IL-15, resulting in the development of the promising cancer drug candidate ALT-803 (15, 16), which is currently undergoing clinical investigation. In yet another approach, de novo computational design algorithms were leveraged to engineer an IL-2/IL-15 mimetic that operates independently of the IL-2Rα and IL-15Rα subunits (17). Countless additional engineered IL-15 molecules are building on the aforementioned strategies to develop fusion proteins with enhanced signal bias, prolonged serum persistence, and/or improved disease targeting. As these strategies mature, we have the unique opportunity to interrogate the biology of IL-15 and to harness structural and biochemical insights to inform cytokine design.

Robinson and colleagues (9) capitalized on their designed PEGylated IL-15 molecule (NKTR-255) to elucidate mechanistic details of IL-15 signaling and to understand how function can be modulated through molecular engineering efforts. Importantly, they observed that in contrast with the unconjugated IL-15 cytokine, NKTR-255 does not substantially utilize trans presentation of IL-15Rα as an activation pathway on either naive or memory $CD8^+$ T cells. Conversely, the authors observed that NKTR-255 relies on cis presentation of IL-15Rα on $CD8^+$ T cells (although not NK cells) as a crucial mechanism for activation, whereas unconjugated IL-15 did not exhibit this dependence (Figure 1). Collectively, the unique behavior of NKTR-255 versus IL-15 illustrates the relevance of chemical modification in biasing cytokine function toward specific cell subsets. Moreover, the divergent effects of NKTR-255 on T cells, which express higher levels of IL-15Rα, compared with NK cells, which express higher levels of IL-15Rβ, suggest that relative expression levels of IL-15Rα versus IL-2Rβ on responsive cells can toggle immune responses to IL-15–based interventions. The nuanced and context-dependent activities of IL-15 agonists will have important implications for clinical advancement of the many IL-15 drug candidates in the pipeline.

Future studies will further clarify the role of soluble and surface-bound IL-15Rα in biasing the activity of NKTR-255 and other IL-15–based therapeutics. Also, although $CD8^+$ T cell responses were explored in detail in this study, the behavior of $CD4^+$ T cells in the context of IL-15 treatment must be further investigated in future studies, particularly since IL-15 sensitivity is heightened on $CD4^+$ T cells in humans compared with mice (2, 18, 19). In addition, the important roles of serum persistence, molecular kinetics, and subcellular trafficking dynamics remain to be defined. The effect of tumor-targeting strategies on IL-15 signaling will also be an important consideration in designing safe and effective therapies that localize to the site of disease. Next-generation strategies may synthesize existing IL-15 engineering approaches, for instance, combining cytokine mutation with chemical modification to further manipulate immune bias. The distinctive lymphocyte-promoting activities of IL-15 therapeutics also make them amenable to synergistic integration with other immunotherapeutic modalities, such as vaccines, adoptive cell transfer, and immune checkpoint inhibition. Overall, the work by Robinson et al. (9) answers important questions concerning the IL-15 system, while also opening up new frontiers to explore in cytokine therapeutic development. It will be exciting to witness the coming of age for NKTR-255 and other such designer IL-15 drugs alone and in combination therapies for a broad range of disease applications.

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