COMPETING FINANCIAL INTERESTS

The author declares no competing financial interests.

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IL-2 and IL-15 signaling complexes: different but the same

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Interleukin 2 (IL-2) and IL-15 use receptors with the same signaling subunits. New structural data show that the signaling complexes they form are topologically nearly identical, which suggests that other factors are responsible for the distinct signaling properties of these complexes.

The largest group of cytokine receptors, the class I receptors, includes three families[1]. These all use a common structural blueprint for initiating signals in receptor-expressing cells that involves the coassociation of pairs of signaling subunits. Each family, however, uses a shared signaling subunit characteristic of each family: the common β-chain, gp130 or common γ-chain (γ_c). IL-2 and IL-15 use γ_c but are unique in also sharing their second signaling subunit, IL-2Rβ. A third, 'private' subunit, IL-2Rα or IL-15Rα, is cytokine specific and stabilizes binding but apparently lacks signaling activity. A key question therefore is as follows: how do IL-2 and IL-15 produce distinct signals using the same pair of signaling subunits, γ_c and IL-2Rβ? Ring *et al.* address this matter in this issue of *Nature Immunology*[2].

Class I cytokine receptors are triggered by cytokine-induced dimerization of their signaling subunits, which are noncovalently associated with tyrosine kinases of the Jak family[3]. This results in phosphorylation and activation of the Jak kinases, which then recruit and phosphorylate members of the STAT family of transcription factors. Finally, the phosphorylated STAT proteins dissociate from the receptors and activate transcription in the nucleus. As the signal-initiating kinases Jak1 and Jak3 and transcription factor STAT5 are activated by the receptors for IL-2 and IL-15, it is no surprise that the functions of IL-2 and IL-15 overlap[4]. Both cytokines stimulate the proliferation of T cells and B cells and enhance the generation and persistence of natural killer cells. However, *Il2*$^{-/-}$ and *Il15*$^{-/-}$ mice have very different phenotypes and, broadly speaking, whereas IL-2 also participates in activation-induced cell death and the maintenance of peripheral regulatory T cells, IL-15 is particularly needed to support the survival of CD8$^+$ memory T cells[4].

Explanations for how the shared γ_c and IL-2Rβ subunits produce different IL-2- and IL-15-dependent signals have focused on the distinct patterns of expression of the α-chains and on the *cis* versus *trans* presentation of IL-2 and IL-15 by the α-chains[4]. Another possibility is that the signaling complexes have divergent topologies that affect the types of signals generated. Published comparisons of IL-15–IL-15Rα and IL-2–IL-2Rα complexes have emphasized their similarities[5,6] and the feasibility of the *trans* presentation of both cytokines[5]. Ring *et al.* now show that the complete receptor complexes formed by IL-15 and IL-2 are effectively identical in their 'business' (signaling) ends and that both complexes are, in principle, able to induce the same signals and the same type of transcriptional remodeling[2]. In doing so, Ring *et al.*[2] both simplify and redefine the conundrum: the specificity of IL-2- and IL-15-associated signaling is either highly context dependent or sensitive to the kinetics of complex assembly or disassembly, an emerging theme in cytokine biology.

This same research group[2] has already determined the structure of the quaternary IL-2 signaling complex[7] and has now completed the IL-15 complex. The two complexes have turned out to be remarkably similar. Loosely speaking, the average distance between equivalent atoms in the complexes is ~1 Å, which is less than the diameter of a single carbon atom. The shared signaling γ_c and IL-2Rβ subunits, which have membrane-distal (D1) and membrane-proximal (D2) domains, assemble into a Y-shaped dimer with the cytokine nestled in a pocket formed by the 'elbow' regions between D1 and D2 (**Fig. 1a**). The only differences are in the slight repositioning of their D1 domains, as the D2 domains of γ_c and IL-2Rβ, which would position the associated kinases just below the membrane, are nearly identical; this rules out the possibility that topological differences are the explanation for the differences in signaling by these receptors. The chemistry of the recognition of IL-2 and IL-15 by γ_c and IL-2Rβ is very different, however. For example, only three of the fifteen IL-15 residues that contact IL-2Rβ are shared by IL-2, and even these are not conserved in sequence; only their positions in three dimensions and the nature of their contacts with IL-2Rβ are preserved. The divergence of the other twelve residues seems to have been unconstrained, in some cases swapping polar contacts for hydrophobic contacts. A similar pattern emerges for the recognition of IL-2 and IL-15 by γ_c. However, the γ_c subunit relies more heavily on hydrophobic contacts and on a smaller interface for its degenerate recognition of IL-2 and IL-15, which obviates the need for pairing of polar or highly charged residues at different, large interfaces.

Having established that the two complexes are extremely similar structurally, Ring *et al.* turn to settling for once and for all the question of whether they are able to produce the same signals[2]. Both indistinguishable profiles as well as distinct differences in signaling

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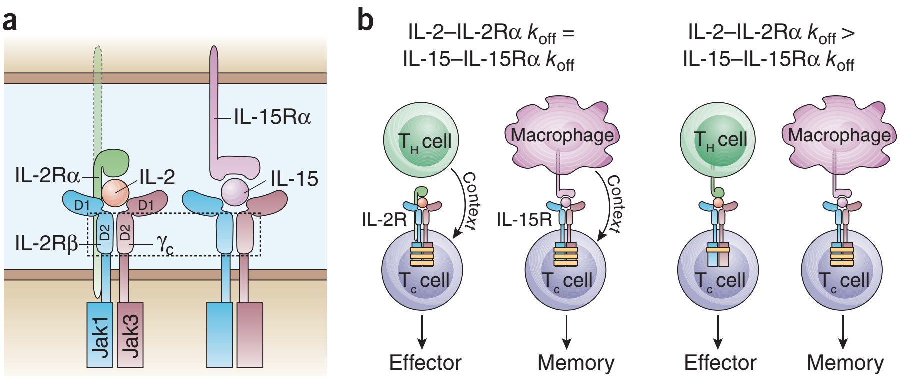

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Figure 1 Signaling by IL-2 and IL-15. (**a**) Signaling complexes formed by IL-2 and IL-15, as determined by Ring *et al.*[2]. The dotted green line indicates the possible *trans* presentation of IL-2 (ref. 5). Box indicates the regions of almost complete identity in both the structure and organization of the D2 domains of the signaling subunits, as now demonstrated[2], which position Jak1 and Jak3. (**b**) Different ways that identical signaling complexes could produce distinct signals in a cytotoxic T cell (T_c cell) interacting with a helper T cell (T_H cell) or macrophage. At left, despite their different architectures, the complexes have similar off rates (k_{off}), which results in a similar amount of phosphorylation and activation of Jak1 and Jak3 (yellow stripes). In this case, the signaling context (that is, the involvement of additional signals, such as that from the T cell antigen receptor) results in distinct signaling outcomes. At right, the two complexes have the same architecture and different off rates; as a result, engagement produces different amounts of activation of the kinases via different degrees of their phosphorylation or of their immediate substrates. (These diagrams are not intended to present the only functions of IL-2 or IL-15.)

kinetics or efficacy have been reported before (as noted by Ring *et al.*[2]). The authors approach this by assessing the effects of IL-2 and IL-15 on cells that lack IL-2Rα and by using soluble IL-15–IL-15Rα complexes to impede the engagement of IL-15Rα or a mutant form of IL-2 that binds strongly to IL-2Rβ in the absence of IL-2Rα. Dose-response studies show that the amount of cytokine needed to produce half-maximal signaling correlates with the affinities of the cytokines and cytokine derivatives for IL-2Rβ. Notably, all the cytokines produce similar amounts of proximal signaling, with the same kinetics, at saturating doses. These experiments show that IL-2 and IL-15 produce essentially identical amounts of signaling, once affinity and the effects of the α-chains are taken into account. In an even more rigorous test of downstream signaling, Ring *et al.* use next-generation sequencing to compare the transcriptional remodeling that accompanies the treatment of splenic $CD8^+$ T-cells with saturating and subsaturating doses of the two cytokines[2]. At saturating doses, the induced gene-expression programs are impressively similar: of the ~5,000 genes regulated by IL-2 or IL-15, >90% have similar expression, as expected for cells receiving very similar or identical signaling inputs. Interestingly, at lower concentrations, the fraction of genes regulated in the same way decreases considerably (to 40–60% after 24 hours).

Given this new work, the case that, for a given degree of engagement in the same cellular context, the receptors for IL-2 and IL-15 signal in exactly the same way must now be considered 'nailed down'. In passing, Ring *et al.* note that the arrangement of the signaling subunits of the type I IL-4 receptor, which are also associated with Jak1 and Jak3, has the same architecture as in the IL-2 and IL-15 receptor complexes[2]. However, signaling by IL-2, IL-15 and IL-4 is not equivalent *in vivo*. The possibilities left are that different amounts of receptor occupancy or the signaling context produce distinct outcomes. The cellular and environmental context is important[4,8], but it is not obvious why distinct receptor complexes would be needed if signaling depends only on when and where the cytokine and its α-chain are expressed, nor is it clear why the receptor-binding chemistries are now so profoundly different. As discussed by Ring *et al.*[2], the relative effectiveness of IL-2 and IL-15 as agonists of IL-2Rβ–γ_c signaling complexes is hard to estimate given the uncertainty over the entropic benefits of *cis* versus *trans* presentation of IL-2 and IL-15 by their α-chains, which might depend on the flexibility of the α-chain[5]. Although unlikely, it is possible that the lower-affinity *cis* binding[9] of IL-2 exactly balances the higher-affinity *trans* binding[10] of IL-15 to its receptor (**Fig. 1b**, left). In this case, differences in signaling would have to be entirely context dependent—for example, requiring additional signals such as those from the T cell antigen receptor. In situations in which the binding activities are not the same—for example, when both IL-2 and IL-15 are *trans* presented (**Fig. 1b**, right)—the higher affinity of IL-15 could lead to enhanced phosphorylation and/or greater activation of receptor-associated Jak kinases. There are already strong hints in the work of Ring *et al.* that subsaturating doses of the cytokines alone lead to differences in gene-expression changes[2].

The importance of the stability of the cytokine-signaling complex, as a form of 'ligand proofreading', has been confirmed for type I interferons[11]. Sixteen type I interferons bind to a single Jak kinase–associated receptor pair (IFNAR1 and IFNAR2), and for the interferons IFN-α2 and IFN-ω, the receptor-bound complexes are nearly identical, which leaves only the stabilities of the complexes able to exert chief control over differences in signaling[11]. Understanding the relative importance of receptor affinity and signaling context will be very important for exploitation of the IL-2Rβ–γ_c complex therapeutically. An additional challenge, as with other ligand-receptor systems[12], will be to understand how cytokine affinity differences really are 'read out' by lymphocytes. Ring and colleagues[2] will probably work all this out as well.

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