Catching a complex for optimal signaling

DOI 10.1074/jbc.H119.010823

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Agonistic antibodies are powerful tools to dimerize receptors in the absence of ligand binding, but high-fidelity receptor activation requires that these antibodies accurately recapitulate the native dimeric state. Spangler *et al.* employ a clever approach to select for antibodies that bind a specific IL-4Rα/γ_c heterodimeric complex in its native signaling conformation, leading to a monovalent "stapler," a single-chain variable fragment (scFv) that binds at the dimerization interface. This powerful approach can be further exploited for a variety of homo- or heterodimeric receptors to achieve signaling, especially in the absence of endogenous ligand.

Cells frequently use transmembrane receptors to convey information from the extracellular environment to the intracellular space. Many of the extracellular ligands that trigger this information exchange do so by mediating receptor oligomerization, sometimes with many complexes possible and with the resultant signal depending on the receptor subunits incorporated. This heterogeneity has often frustrated efforts to develop ligand mimics that can selectively activate one signaling pathway, as the molecular requirements for binding different complexes are often highly overlapping. Antibodies have been developed that can induce receptor dimerization, but the location of the epitope that the antibodies target generally does not lead to cross-linking of receptors in their native "activated" arrangement (1). Because the geometry of the dimerized receptor pair greatly influences their activity, a better strategy is needed to obtain functional antibodies.

Spangler *et al.* (2) now report just such an approach, using a stringent selection process to identify antibodies that only bind to a preformed complex. The authors focus on two cases within the immune system, which is a prime example in which ligand-induced activation of specific signaling pathways controls cellular fate in the form of the expression of genes necessary for cell proliferation, migration, and ultimately immune activation (3–6). In particular, cytokines—critical ligands for lymphocyte homeostasis—generally activate their cognate receptors by heterodimerization or heterotetramerization. Interleukin-4 (IL-4)[2] is a cytokine that binds to the IL-4 receptor α-chain (IL-4Rα) and the common γ-chain (γ_c), leading to heterodimerization in a precise topological arrangement. This in turn results in activation of Janus kinases that are associated with the receptor intracellular domains, leading to transcriptional activation of target genes (7). IL-4 can also bind to IL-4Rα/IL-13Rα1 complexes, leading to different signaling outcomes. Another cytokine, IL-2, can similarly bind to either of two complexes, one containing the IL-2Rα, IL-2Rβ, and γ_c chains (the high-affinity complex) and the other containing only the IL-2Rβ and γ_c chains (the intermediate affinity complex). IL-2Rα itself does not signal, but its expression level appears to correlate with IL-2 sensitivity of the cells. Which complex is formed depends on the differential expression of IL-2Rα on distinct cell types, adding further complications to the system.

The approach from Spangler *et al.* cleverly targets these two cases in a way that overcomes the limitation of traditional antibody-mediated receptor dimerization, by trapping the IL-4 receptor heterodimer in its native signaling state before screening a naive antibody library for high-affinity binders (2). They began by using yeast display of an IL-4 cytokine–receptor complex as bait for a diverse human single-chain variable fragment (scFv) library. Importantly, the IL-4 molecule used was an engineered variant called "Super-4" with 3,700-fold higher affinity for γ_c (8), generating a highly stable ternary complex that would more robustly enable screening. The authors performed several rounds of positive selection against the ternary complex combined with negative selection against the individual components, leading to the generation of three "stapler" scFvs that target the active signaling complex. The best variant (named the IL-4 stapler) was determined to have a binding affinity of 880 nM, more than 10-fold improved over the interaction with the IL-4Rα receptor alone. Furthermore, these scFvs did not bind to the IL-13 or the IL-2 receptor complexes, confirming their receptor type specificity.

To understand the basis for this selectivity, Spangler *et al.* solved the crystal structure of the IL-4 stapler bound to the ternary IL-4 receptor complex. The authors observed that a single IL-4 stapler binds to a composite epitope formed by IL-4Rα and γ_c at the stalk region of the IL-4Rα and γ_c heterodimer near the membrane, with similar contributions from both individual receptors. Moreover, residues from both the heavy and light chain of the IL-4 stapler similarly bound with almost equal contributions, suggesting that they both play pivotal roles in antigen binding and discrimination. Comparison with the crystal structure of the ternary complex in the absence of the IL-4 stapler (9) demonstrated only minimal structural perturbation of the receptor geometry, suggesting that overall

The author declares that he has no conflicts of interest with the contents of this article.

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[2] The abbreviations used are: IL, interleukin; γ_c, common γ-chain; scFv, single-chain variable fragment.

strategy worked and that the IL-4 stapler indeed trapped the ternary complex in its native signaling orientation.

Given the success in generating the IL-4 stapler, the authors turned to the IL-2 system. If the authors could identify a stapler for the intermediate-affinity complex, this could create new opportunities to increase IL-2 sensitivity in cells that do not express IL-2Rα at high levels. In this case, the authors used the binary IL-2/IL-2Rβ complex as their bait, leading to initial hits that were affinity-matured into a final construct termed the amIL-2B stapler. This construct bound the IL-2 complex with an affinity of 15 nM and in an orientation that did not compete with γ_c binding, enabling the subsequent addition of this chain to the complex. In addition, the IL-2B stapler increased the affinity of the interaction between IL-2 and IL-2Rβ by 15-fold. Nevertheless, because IL-2 signaling is generally of high potency, amIL-2B did not potentiate cytokine signaling with WT IL-2. However, when an impaired IL-2 variant was used, STAT5 signaling was increased over 20-fold using the amIL-2B stapler.

The study by Spangler *et al.* nicely demonstrates a potentially universal approach of trapping heterodimeric cytokine receptor complexes in a native state to potently activate signaling. It will be important to confirm that the energetics of the IL-4 stapler determined *in vitro* lead to a functional impact in intact cells and/or *in vivo*, especially considering that excessive IL-4 signaling can promote allergic reactions. Similarly, it will be interesting to see what the *in vivo* consequences are for the amplification in cell signaling observed with amIL-2B; will this particular molecule or others derived using this approach lead to robust activation of cells that do not express IL-2Rα? Would the use of a preformed trimeric complex (including IL-2, IL-2Rβ, and the γ_c chain) lead to a substantially different stapler? Finally, it will be exciting to see what other classes of receptors can be targeted using this approach or whether this approach can be modified to target tumor necrosis factor receptors, co-stimulatory receptors that are expressed on T cells and are activated upon ligand-induced homotrimerization, rather than heterodimerization.

In either case, because the use of cytokines as immunotherapeutic drugs is not always feasible, due to their short half-life or their high potency and subsequent toxicity, cytokine staplers could be an alternative framework for the design of next-generation immunotherapeutics.

References

1. Zhang, P., Tu, G. H., Wei, J., Santiago, P., Larrabee, L. R., Liao-Chan, S., Mistry, T., Chu, M. L., Sai, T., Lindquist, K., Long, H., Chaparro-Riggers, J., Salek-Ardakani, S., and Yeung, Y. A. (2019) Ligand-blocking and membrane-proximal domain targeting anti-OX40 antibodies mediate potent T cell-stimulatory and anti-tumor activity. *Cell Rep.* **27,** 3117–3123.e5 CrossRef Medline
2. Spangler, J. B., Moraga, I., Jude, K. M., Savvides, C. S., and Garcia, K. C. (2019) A strategy for the selection of monovalent antibodies that span protein dimer interfaces. *J. Biol. Chem.* **294,** 13876–13886 CrossRef Medline
3. Bromley, S. K., Burack, W. R., Johnson, K. G., Somersalo, K., Sims, T. N., Sumen, C., Davis, M. M., Shaw, A. S., Allen, P. M., and Dustin, M. L. (2001) The immunological synapse. *Annu. Rev. Immunol.* **19,** 375–396 CrossRef Medline
4. Mellado, M., Rodríguez-Frade, J. M., Mañes, S., and Martínez-A, C. (2001) Chemokine signaling and functional responses: the role of receptor dimerization and TK pathway activation. *Annu. Rev. Immunol.* **19,** 397–421 CrossRef Medline
5. Wang, X., Lupardus, P., Laporte, S. L., and Garcia, K. C. (2009) Structural biology of shared cytokine receptors. *Annu. Rev. Immunol.* **27,** 29–60 CrossRef Medline
6. Croft, M., Benedict, C. A., and Ware, C. F. (2013) Clinical targeting of the TNF and TNFR superfamilies. *Nat. Rev. Drug Discov.* **12,** 147–168 CrossRef Medline
7. Nelms, K., Keegan, A. D., Zamorano, J., Ryan, J. J., and Paul, W. E. (1999) The IL-4 receptor: signaling mechanisms and biologic functions. *Annu. Rev. Immunol.* **17,** 701–738 CrossRef Medline
8. Junttila, I. S., Creusot, R. J., Moraga, I., Bates, D. L., Wong, M. T., Alonso, M. N., Suhoski, M. M., Lupardus, P., Meier-Schellersheim, M., Engleman, E. G., Utz, P. J., Fathman, C. G., Paul, W. E., and Garcia, K. C. (2012) Redirecting cell-type specific cytokine responses with engineered interleukin-4 superkines. *Nat. Chem. Biol.* **8,** 990–998 CrossRef Medline
9. LaPorte, S. L., Juo, Z. S., Vaclavikova, J., Colf, L. A., Qi, X., Heller, N. M., Keegan, A. D., and Garcia, K. C. (2008) Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* **132,** 259–272 CrossRef Medline