Exploiting a Natural Conformational Switch to Engineer an Interleukin-2 Superkine

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Abstract

The immunostimulatory cytokine interleukin-2 (IL-2) is a growth factor for a wide range of leukocytes, including T cells and natural killer (NK) cells [1-3]. Considerable effort has been invested using IL-2 as a therapeutic agent for a variety of immune disorders ranging from AIDS to cancer. However, adverse effects have limited its use in the clinic. On activated T cells, IL-2 signals through a quaternary "high affinity" receptor complex consisting of IL-2, IL-2Rα (termed CD25), IL-2R β, and γ_c [4-8]. Naïve T cells express only a low density of IL-2R β and γ_c, and are therefore relatively insensitive to IL-2, but acquire sensitivity after CD25 expression, which captures the cytokine and presents it to IL-2Rβ, and then γ_c. Here, using in vitro evolution, we eliminated IL-2's functional requirement for CD25 expression by engineering an IL-2 "superkine" (termed super-2) with increased binding affinity for IL-2Rβ. Crystal structures of super-2 in free and receptor-bound forms showed that the evolved mutations are principally in the core of the cytokine, and not participating in direct IL-2Rβ contacts. Rather, molecular dynamics simulations indicated that the evolved mutations in super-2 locked a flexible helix in the cytokine's IL-2R β binding site into a position similar to a conformation seen when IL-2 is bound to CD25. The evolved mutations in super-2 also recapitulated the functional role of CD25 by enabling potent phosphorylation of STAT5 and vigorous proliferation T cells irrespective of CD25 expression. Compared to IL-2, super-2 induced superior expansion of cytotoxic T cells, leading to improved anti-tumor responses in vivo. Importantly, super-2 elicited proportionally less expansion of T regulatory cells and reduced pulmonary edema, the principal dose-limiting adverse effects of IL-2 in the clinic. Collectively, we show that invitro evolution has captured a natural structural mechanism that enhances IL-2 potency and regulates target cell specificity, which has implications for immunotherapy.