

IMMUNOLOGY

Evolving superkines for selectivity

Combinatorial protein engineering based on structural data and the differential expression of alternate second receptor chains for interleukin-4 (IL-4) is used to modify and tune cellular specificity on primary human cells.

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Cytokines have a central role in the modulation of immune and inflammatory responses, and thus there is intense interest in using these secreted signaling molecules as therapeutics in a host of pathological milieus, including infectious, autoimmune and neoplastic diseases. However, the pleiotropy and redundancy that is inherent in cytokine signaling has limited their application because of side effects that probably arise from activities elicited from different cell types. In this issue, Juntilla *et al.*¹ attempt to circumvent this difficulty by using combinatorial protein engineering to develop cell type-specific IL-4 variants.

IL-4 is an immunomodulatory cytokine that regulates both B- and T-cell responses by acting as a central promoter of T helper type 2 (T_H2) cell formation and B-cell activation through interaction with the type I (IL-4R α / γ c) heterodimeric receptor. IL-4 also interacts with an alternate type II heterodimer (IL-4R α /IL-13R α 1) that is expressed primarily on nonhematopoietic cells, where it can inhibit cell proliferation, transduce signals through Jak and STAT effectors and promote inflammation^{2,3}. The ability of IL-4 to induce responses from both hematopoietic and nonhematopoietic cells may give rise to toxicity and limit its clinical utility^{4,5}. Although many cytokines have shown promise in the treatment of disease⁶, dose-limiting toxicity remains a pressing issue that limits the efficacy of cytokine therapies⁷. Previous studies evaluating the efficacy of IL-4 in the treatment of malignancies have fallen short of expectations⁸, and IL-4 is not currently approved for clinical use despite data that supports a central role for the cytokine in the differentiation of T_H2 cells from CD4⁺ T-cell progenitors⁹.

Juntilla *et al.*¹ extend previous attempts to engineer IL-4 (ref. 10) and investigate the apparent disparity that arises between the striking increases in affinity obtained through combinatorial selections and the relatively modest increases in biological activity. Through computational simulation of receptor expression and occupancy in combination with experimental investigation of cells with differential

expression of alternate receptors, the authors advance a key hypothetical assertion: they propose that engineered IL-4 variants are most effective when expression of the second receptor is low in comparison to that of the primary receptor. This goes beyond simply engineering IL-4 variants that preferentially interact with and activate type I or type II receptors to a detailed assessment of the biological consequences of these changes. This is an important advance because it combines protein engineering with cell biology and physiology, and in our opinion, studies of this type will be essential to extend structural knowledge to therapeutic applications. Although the authors demonstrate a viable structure-based approach to the directed evolution of cytokines with selective cellular activity, an important impact of their work is that it provides a clear and testable hypothesis to use when evaluating the bioactivity of other engineered cytokines. In other words, this work poses an important question:

can differences in the relative expression of cell-surface receptors be exploited to tailor selectivity even when alternate receptors are not used, such as in the case of receptors for type I interferons (IFNs) and most other interleukins?

Although other cytokines may use alternate receptor systems that could be amenable to this engineering approach in a way analogous to the IL-4 system, this has not yet been well established. Nevertheless, there are several other therapeutically relevant cytokines that rely on a small number of archetypical multimeric signaling complexes that may benefit from the framework of this investigation. In light of this, it is possible that the success of any future attempt to engineer cytokines for a desired cell selectivity and reduced cytokine toxicity will be determined in part by whether these receptor partners are expressed coordinately at constant ratios on all cells or are independently expressed on different cells types at varying relative

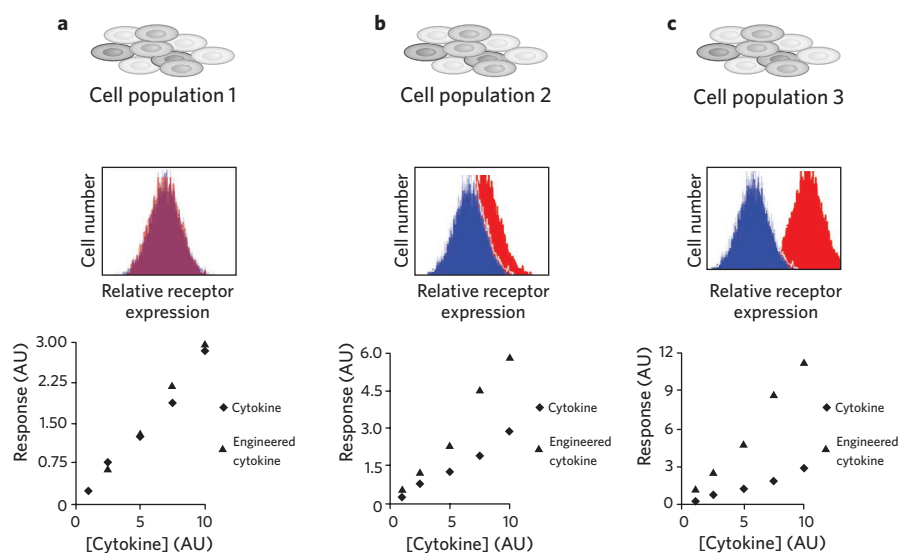


Figure 1 | Hypothetical influence of relative receptor expressions on the activity of engineered cytokines. (a–c) Three populations of cells are shown illustrating receptor subunits expressed at parity (a), receptor subunits that have modest expression differentials (b) and receptor subunits that have marked expression differentials (c). According to the hypothetical framework advanced by Juntilla *et al.*¹, increases in cytokine affinity for its cognate receptor could be influenced by relative expressions of receptor subunits whereby changes in cellular responses would be most apparent in cases where a differential exists. AU, arbitrary units.

expressions of receptor in a manner that can be rationally exploited (Fig. 1). If this is indeed the case, it may provide concrete inroads to using engineered cytokines effectively for the treatment of a variety of diseases while limiting their negative effects. For instance, type I IFNs are also used as immunomodulatory therapeutics but have a similar limiting toxicity. Their receptors are widely expressed, and IFN ligands interact with and induce assembly of this target heterodimeric receptor by binding first to the high-affinity and then to the low-affinity receptor subunit. Although it is known that there is considerable variation in type I IFNAR receptor expression and this can influence cell fate after IFN treatment¹¹, there has

been little systematic investigation into the relative expressions of each subunit on varying cell types. It is exactly this sort of investigation that we believe could further inform rational efforts to engineer selective cytokines that enhance clinical benefit while limiting toxicity, and is highlighted by the experiments described by Juntilla *et al.*¹.

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Competing financial interests

The authors declare no competing financial interests.