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Engineered IL-10: a matter of affinity

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Abbreviations: DCs, dendritic cells; IBD, inflammatory bowel disease; IL, interleukin; IL-10R, IL-10 receptor; ILCregs, regulatory innate lymphoid cells; ILCs, innate lymphoid cells; IFN- γ , interferon- γ ; MET, methionine MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells; STAT, signal transducer and activator of transcription; TYR, tyrosine.

Interleukin (IL-)10 plays an important immunoregulatory role due to its ability to elicit anti-inflammatory responses on innate and adaptive immune cells.¹ Discovered in 1989 by Fiorentino *et al.*, IL-10 was described as a Th2-cell-derived cytokine that could suppress cytokine synthesis in Th1 cells.² Later on, it was demonstrated that IL-10 could be produced by other cells including regulatory T and B cells,³ macrophages, dendritic cells (DCs) and innate lymphoid cells (ILCs).⁴ IL-10 has been studied in different fields, including immune tolerance to allergens and to infectious agents, organ transplantation, and oncology.⁵

For decades, regulatory T and B cells have been considered the main source of IL-10. However, there is growing evidence on the relevance of ILCs as IL-10 producing cells (ILCregs). Indeed, they have been shown to suppress Th2 and ILC2 cells following allergen immunotherapy.⁴ Among other mechanisms, IL-10 reduces the antigen-presenting capacity of innate cells by decreasing the expression of major histocompatibility complex (MHC) and costimulatory molecules (CD80/CD86).⁶ IL-10 also inhibits the secretion of pro-inflammatory cytokines by T cells,⁷ monocytes, and macrophages.^{8,9}

Despite its potent biological role, IL-10 administration has only elicited low/mild anti-inflammatory effects in several clinical trials to treat inflammatory bowel disease (IBD).⁸ This low efficacy has been attributed to the difficulty for IL-10 to reach the target tissue.⁸ Furthermore, IL-10 can promote the cytotoxic function of CD8⁺T cells by increasing interferon- γ (IFN- γ) and granzyme B production. Thus, the proinflammatory activity of IL-10 may have counteracted its therapeutic effects for the treatment of different inflammatory diseases.^{8,9} The dual function of IL-10 pertaining to its immune suppressive and immune-stimulatory effects remains unclear, and recent studies on its structure and functionality have shed light on this matter.^{8,9}

IL-10 is a dimeric protein that binds to the IL-10 receptor (IL-10R), which is comprised of two subunits of IL-10R α and two of IL-10R β . The IL-10 dependent dimerization of IL-10R α and IL-10R β , promotes the activation of signal transducer and activator of transcription (STAT) 1 and 3, regulating the immunomodulatory function of IL-10.⁹ Saxton *et al.* elucidated the complete molecular structure of the IL-10R complex by engineering IL-10 variants with different affinity for IL-10R β that upon binding form the hexameric IL-10–IL-10R α –IL-10R β complex.⁹ The affinity of IL-10 for IL-10R α is stronger than for IL-10R β . For this reason, it has been proposed that the interaction between IL-10 and IL-10R β dictates the type of biological effect elicited by IL-10. In this context, Gorby *et al.* observed that an IL-10 variant with

increased affinity for IL-10R β triggered a stronger signaling and activation of STAT1/3 in monocytes and CD8⁺ T cells, compared to its native form. Further research is warranted to determine IL-10 variants activity in inflammatory environments.⁸

Using IL-10 variants, Saxton *et al.* observed a hydrophobic interaction between tyrosine (Tyr) in the loop 3 from the domain 1 of IL-10R β and methionine (Met) of IL-10 α 1.⁹ Interestingly, they reported that IL-10 variants with a higher affinity to IL10R β had mutations adjacent to the Met of IL-10, enhancing the hydrophobicity of this region. Moreover, they conducted a comprehensive analysis of IL10/IL-10R β interaction and STAT1/STAT3 activation on different cell populations, demonstrating that IL-10 signaling is limited by the expression of its receptor. However, this could be compensated by enhancing its affinity for IL10R β .⁹

The engineered IL-10 variants showed an anti-inflammatory function similar to the native form. They inhibited the secretion of IL-1 β , IL-6, TNF- α , IL-8, and IFN- γ in PBMCs and macrophages stimulated with endotoxin or anti-CD3. In addition, they downregulated CD86 and MHC-II expression on monocytes. As expected, the IL-10 variants induced an enrichment of pro-inflammatory genes (*IL9*, *IL17F*, and *GZMB*), and downregulated the expression of chemokine related genes (*CCL1*, *CCL17*, and *CCL22*) on CD8⁺T cells, thus promoting cytotoxicity. Remarkably, IL-10 variants with a stronger affinity for IL10R β maintained this pro-inflammatory effect on CD8⁺T cells, whilst IL-10 variants with a lower affinity for IL10R β reduced the pro-inflammatory activity of these cytotoxic cells.

With their findings, Saxton *et al.*, proposed that IL-10's double-edge-sword is controlled by the expression of IL-10R but also by the affinity of IL-10 to the subunit IL-10R β . Engineered IL-10 variants with lower affinity for IL-10R β maintain their anti-inflammatory function on monocytes and macrophages, however, they reduce their pro-inflammatory effects on CD8⁺T cells. This study contributes to the development of IL-10-based treatments in allergic and autoimmune diseases. The winding journey of IL-10 as an exogenous immune regulator is not over yet and further studies are warranted to improve our understanding of the potential therapeutic effect of novel engineered IL-10 variants.

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