Deep immune cell profiling of patients with defects in the leptin-melanocortin signaling pathway

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The leptin-melanocortin signaling pathway plays an important role in body weight regulation and defects in this pathway can lead to rare monogenic forms of obesity. The most common defects include missense or nonsense mutations of the leptin receptor (LEPR), proopiomelanocortin (POMC), and the melanocortin-4 receptor (MC4R). While the effects of these mutations on the metabolism have been extensively studied, their role in immune regulation remains elusive.

To characterize the impact of the leptin-melanocortin signaling cascade on immune cell composition and function we collected a cohort of three patients with heterozygous mutations in *MC4R*, four patients with homozygous or compound-heterozygous mutations in *LEPR*, and six patients with homozygous mutations in *POMC*, as well as control groups of age- and sex-matched lean and obese individuals. Peripheral blood mononuclear cells (PBMCs) were isolated from all subjects and stimulated ex vivo for 4 h with ionomycin/PMA or lipopolysaccharide (LPS). Samples were barcoded and pooled and subsequently stained with a panel of 35 markers and analyzed by mass cytometry. The R packages CATALYST and diffcyt were used to perform a detailed analysis of B cells, T cells, myeloid cells, and NK cells, which were subsequently compared between groups.

In patients with *LEPR* mutations, we detected a reduced frequency of pro-inflammatory IL-8$^+$ IL-6$^+$ TNFα$^+$ monocytes, whereas in patients with *MC4R* mutations we found an increased abundance of B cells. Patients with *POMC* mutations displayed increased frequencies of TNFα$^+$ IFNγ$^+$ CD4$^+$ and CD8$^+$ T cells as well as a significantly increased expression of TNFα and IFNγ in T cells.

Our study provides an in-depth characterization of PBMCs of patients with rare monogenic forms of obesity and helps to elucidate the role of LEPR, POMC and MC4R in immune regulation and function. Additional functional assays are currently being performed to validate our mass cytometry findings.