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Modelling of long-term macrophage differentiation in vitro

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Tissue macrophages lifespan can range from weeks to years. In pathological conditions such as cardiac inflammation or cancer, monocyte-derived macrophages undergo prolonged differentiation, and can adopt pathological programs. Most of previous research primarily focused on macrophage responses to short acting stimuli, while long-term macrophage differentiation and functional programming was insufficiently explored. This thesis aimed to establish a reliable in vitro platform for investigating the prolonged differentiation process of macrophages derived from human monocytes. Specific aims included: 1) to establish conditions under which primary human monocyte-derived macrophages can differentiate over 24 days; 2) to analyze expression of genes that define the proinflammatory programming macrophages during their long-term differentiation; 3) to analyze scavenger receptors that define scavenging abilities of macrophages; 4) to examine the expression of metabolic regulators during long-term macrophage differentiation; 5) to examine α-SMA and Ki-67 as indicators of macrophage trans-differentiation and the transition from a proliferative to a non-proliferative phenotype. The final aim was to identify transcriptome differences between early and late stages of macrophage differentiation. To establish the model, CD14+ human monocytes were differentiated under 2 conditions: M-CSF+IL-4, and M-CSF+IL-4+TGF-β. The combination of M-CSF + IL-4 resulted in the longest survival of macrophages in culture: up to 36 days. The expression of 22 genes, categorized into seven functional groups, was analyzed by RT-PCR during macrophage differentiation. These groups included cytokines, regulators of migration and recruitment, extracellular matrix modulators, chitinase-like proteins, scavenger receptors, metabolic regulators, and markers associated with the macrophage-tomyofibroblast transition (MMT). IL-4 alone and IL-4 in combination with TGF-β were used as stimuli in the long-term differentiation model. When IL-4 was combined with TGF-β, it had a more pronounced effect on the expression of genes linked to chronic inflammation in macrophages, compared to IL-4 alone. This combination strongly increased the expression of IL1RN, counteracting the increase in proinflammatory IL1B, and also upregulated genes involved in inflammation and fibrosis, including FN1, CHI3L1, CHI3L2, OLR1, AKR1C3, SLC16A3, and ACTA2. Exposure to LPS, used to test the inflammatory reactivity of macrophages, triggered an increase in the expression of genes associated with inflammation, such as IL1B and IL1RN, migration and recruitment receptors such as CCR1 and CCR5, and metabolic reprogramming markers such as PFKFB3, an enhancer of aerobic glycolysis. Full transcriptome analysis was performed by NGS on macrophages differentiated under M-CSF+IL-4 stimulation for 6 and 36 days. The total number of upregulated genes on day 36 was 1356; the total number of suppressed genes was 1003. Five most pronounced upregulated gene families on day 36 included aminoacyl-tRNA synthetases, lysosomal proteins, transporters, transcription factors, and aldehyde dehydrogenase. Five most suppressed gene families on day 36 were cholesterol biosynthesis pathway genes, solute carrier family, G protein-coupled receptors, integrins and adhesion molecules, and fatty acid and lipid metabolism genes. These changes reflect the adaptation of macrophages to their environment, either by promoting inflammation and tissue damage or by modifying their metabolic state to maintain cellular functions under challenging conditions. This analysis allowed the identification of several biomarkers of long-term macrophage differentiation in the context of IL-4 cytokine, including CHIT1, CHI3L1 and GDF15 that can be used for histological analysis in chronically inflamed or tumor tissues. Confocal microscopy analysis showed that macrophages stimulated with IL-4 at later stages of differentiation had the capacity to divide and to transdifferentiate into myofibroblast-like cells, which are key drivers of pathological remodeling and fibrosis. In summary, our model provides a useful tool to dissect macrophage behavior under prolonged stimulation. TGF-β, in particular, was found to drive expression patterns linked to chronic inflammatory states and pathologies such as fibrosis and cancer. This long-term culture model is crucial for studying the persistent inflammatory processes and functional alterations in macrophages over extended periods.