



LEPTIN MODULATES MACROPHAGE PHENOTYPE THROUGH A mTORC2 DEPENDENT MECHANISM

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Obesity affects millions of people worldwide and causes a systemic low-grade inflammation and adipose tissue (AT) immune cell infiltration. Leptin is an adipokine produced by AT in response to food intake, inducing satiety and energy expenditure in the hypothalamus. Leptin has also been shown to have pro-inflammatory activity. Obese individuals have elevated leptin serum levels and increased pro-inflammatory (M1) AT macrophage (ATM) numbers, which is associated with a hypothalamic resistance to leptin. In neurons, leptin activates both the STAT3 and the PI3K/AKT/mTOR pathways. mTOR is a structure with two protein complexes (mTORC1 and mTORC2) which sense cellular nutritional state. The main component of mTORC1 is the protein Raptor and, for mTORC2, Rictor. These complexes sense energy levels in the cell and nutrient concentration in the environment, and can influence the overall cell metabolism. Studies show that mTORC1 is linked to the M1 phenotype, while mTORC2 has been implicated with the anti-inflammatory (M2) phenotype, associated with tissue repair.

Some of our initial results show that leptin signaling within the cell is linked to mTORC2 activity. We hypothesized that leptin acts through the mTOR pathway as a systemic nutritional sensing hormone to inform immune cells, such as macrophages, on the availability of nutrients. Our aim is to pinpoint the importance of leptin/mTOR for macrophage function and metabolism.

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To determine the effects of leptin on macrophage cytokine production and to evaluate whether it is linked to the mTOR pathway, we used bone marrow-derived macrophages (BMDMs) from wild type (WT) mice and treated them with rapamycin, an mTOR inhibitor. Macrophages pre-treated with rapamycin prior to LPS secreted reduced levels of proinflammatory cytokines, in comparison to those that were left untreated. Thus, we concluded that the effects of leptin were dependent on mTOR activity.

We used bone marrow derived macrophages from RaptorLoxp x LyzMCre (Raptor KO) and RictorLoxp x LyzMCre (Rictor KO) mice, thus eliminating the activity of mTORC1 and mTORC2, respectively, in these cells. BMDMs were treated with leptin prior to LPS stimulation. Leptin enhanced the effect of LPS, leading to increased proinflammatory cytokine production (measured by ELISA assays and flow cytometry), alongside expression of genes associated with the production of said cytokines (measured by qPCR). This effect was abrogated in Rictor KO macrophages, indicating that leptin effects are mTORC2 dependent.

Furthermore, to understand the effects of leptin on mitochondrial function in BMDMs via the mTORC2 pathway, we also used Rictor KO BMDMs to evaluate mitochondrial dysfunction by flow cytometry. Mitochondrial dysfunction is a hallmark of M1 macrophages, as reactive oxygen species (ROS) production in these cells is exacerbated in a context of inflammation, which causes damaging mitochondrial stress. We observed a decrease in dysfunctional mitochondria in Rictor KO BMDMs in comparison to those with normal mTORC2 activity. This further supports our hypothesis that leptin exacerbates the inflammatory activity of macrophages through a pathway dependent on mTORC2.

In summary, our results show that mTORC2 deficiency in macrophages decreases cytokine expression and production, along with a reduction in mitochondrial dysfunction. Therefore, we suggest that the proinflammatory effects of leptin on macrophages depends on mTORC2 activity.