ImmunoTools *special* Award 2014

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Immunometabolistic phenotyping of macrophage specific Rictor-KO mice

In the past decades, obesity and its associated diseases have become an enormous socioeconomic problem in the world. Obesity is a strong risk factor for the development of insulin resistance, glucose intolerance and a raise in blood pressure and triglycerides, a condition in the body that is known as metabolic syndrome. The metabolic syndrome is a precursor for a lot of severe diseases, such as Type II diabetes and atherosclerosis [1-3]. In the past years, accumulating evidence supports a role of macrophages in the onset of the metabolic syndrome [4]. A lot of effort was done describing the activation state of these macrophages in the adipose tissue and it was recognized that not only macrophages but also other cells of the innate and adaptive immune system contribute to the inflammatory state in adipose tissue that mediates insulin resistance [5].

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase with a molecular weight of 290 kDa and has important functions in survival, metabolism, growth, proliferation, and aging of a cell [6-8]. mTOR is part of two distinct complexes: mTORC1 (mTOR complex 1) and mTORC2. mTOR plays an important function in the regulation of immune responses in macrophages [9,10]. Moreover, tissue-specific deletion of mTOR components revealed that both mTORC1 and mTORC2 are involved in the regulation of whole body metabolism and that disturbances in this signalling pathway can lead to metabolic disorders [11,12].

In the context of the complex interplay between the immune system and metabolism and the important functions of mTOR in both immune regulation and control of metabolism, my goal is to investigate the role of mTORC2 in macrophages and its associated implications for the whole body metabolism. For this purpose, we

generated mice that have a macrophage specific knockout of Rictor, which is a protein specific for the mTORC2 complex. These mice are homozygous for a loxP cassette in the Rictor gene and have the cre-recombinase under the control of the lysozyme M promoter.

First of all, the package from ImmunoTools will help us to get murine macrophages out of the bone marrow (by differentiating isolated bone marrow in the presence of M-CSF). Furthermore, we will analyze the activation state of these macrophages by polarizing them in the presence of LPS+IFNgamma (M1 macrophage) or IL-4 (M2 macrophage). After these in-vitro analyses, we are going to characterize the “in-vivo phenotype” of the macrophages in the different mouse tissues and the consequences of macrophage specific Rictor knockout on the activation state of the other immune cell compartments. Therefore, the antibodies from ImmunoTools would help us a lot with our flow cytometric analyses in order to distinguish the different immune cell compartments and have a closer look at the complex interplays between the immune cells in the context of low grade inflammation, which is one of the hallmarks of obesity.

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