Abstract

Chronic inflammation associated with obesity is an important factor in the pathophysiology of insulin resistance. This inflammation is supported by alterations in the cellular composition of adipose tissues. Macrophages are crucial immune cells in adipose tissues during obesity.

During steady and lean state, macrophages residing in the tissues display M2 phenotype, which secreting anti-inflammatory cytokines and thus maintains metabolic homeostasis and insulin sensitivity. Obesity increases the proportion of macrophages in the adipose tissues and adopts M1 phenotype, secrete proinflammatory cytokines and contribute to chronic inflammation seen in obese individuals.

Recent studies have identified distinct subpopulations of macrophages within adipose tissues from obese individuals. These Adipose Tissue Macrophages (ATMs) are described as Metabolically Activated Macrophages (MMe). Although these macrophages secrete proinflammatory cytokines but are distinct from M1 macrophages and are crucial in metabolic alterations in adipose tissues.

To better understand the characteristics and roles of MMe macrophages, we have established an *in vitro* model of MMe using THP-1 cell line. We used this model to investigate inflammatory patterns, surface markers and metabolic features and compared them with M1 and M2. We observed that MMe had lipid accumulation in its cytoplasm. MMe displayed distinguishing surface markers from M1 and M2. Surface markers exhibited by MMe were related to lipid metabolism like CD36, PLIN2, FABP4 and ABCA1. MMe also secrete proinflammatory cytokines like TNFα, IL6, IL1β and IFNγ, albeit to a lesser extent compared than M1. This metabolic activation and inflammation leads to ER stress and altered lysosomal function. Due to lipid stress, MMe exhibit impaired autophagy.

Unlike M1 and M2, which are dependent on glycolysis and oxidative phosphorylation respectively, MMe exhibited unique metabolism. MMe had upregulated lipid metabolism in comparison to M1 as indicated by increased expressions of PPARγ and PGC1α. However, glycolysis was subdued compared to M1. Both glycolysis and fatty acid oxidation is important for their inflammatory phenotype unlike in M1 where glycolysis seems to be more important.

To understand the interaction between macrophages and adipocytes, conditioned media (CM) from macrophages was introduced to differentiated adipocytes. MMe reduced the expressions of adiponectin in adipocytes. Both M1 and MMe CM reduced the expressions of adiponectin as well as pAkt levels, while M1 additionally impaired the adipocyte differentiation. This indicates that both these subclasses of macrophages affect adipocytes differently.

Untargeted proteomics data from mass spectrometry clearly distinguished the MMe from M1 and M2. We found that M1 are important in infection and pathogen killing activity, while M2 has an important role in wound healing and resolving inflammation. MMe are characterized with increased peroxisomal lipid metabolism and combating oxidative stress, which are crucial processes during obesity to restore homeostasis.

This cell based *in vitro* model of MMe effectively replicates the features of ATMs that are present *in vivo*. It can be a valuable tool to investigate various characters of ATMs and to study the macrophage adipocyte interactions. This can further help in screening potential targets for obesity and insulin resistance.