Abstract

Despite the availability of effective therapy of hypercholesterolemia and hypertension, cardiovascular mortality continues to be very high in the Western world. Inflammatory changes occurring in the arterial wall as well as in the adipose tissue play a major role in the development of atherosclerosis. Macrophages are involved in the process of atherogenesis as early as atherosclerosis begins to develop, when, still as monocytes, they migrate and adhere to the arterial wall as a result of endothelial activation and stimulation by pro-inflammatory substances. Adipose tissue has long been recognized as an important endocrine organ, with part of adipose tissue made up by a large amount of macrophages capable of producing a large number of pro-inflammatory cytokines, which contribute to the development of low-grade chronic inflammation important in the development of atherosclerosis.

In samples of subcutaneous, visceral and perivascular adipose tissue (SAT, VAT, and PVAT, respectively) obtained from healthy subjects (living kidney donors, LKD), we analyzed macrophages and their polarization, gene expression of pro-inflammatory cytokines and the effect of substances released by VAT on the level of monocyte adhesion to the endothelium. In some analyses, we included samples of SAT, VAT and PVAT obtained from patients with angiography-documented peripheral arterial disease (PAD). Results were compared both between the groups of healthy and diseased subjects and between individual types of adipose tissue (SAT, VAT, PVAT); in addition, we sought to analyze their relationship to cardiovascular risk factors.

Adipose tissue samples of the PAD group exhibited significantly higher gene expression of all the pro-inflammatory genes assessed, most significantly in VAT; however, otherwise no significant differences were found between the individual types of adipose tissue or their relation to cardiovascular risk factors. The products of VAT analyzed using adipose tissue-conditioned media (ATCM, obtained by incubation of VAT in a culture medium *in vitro*) significantly increased the level of monocyte adhesion to the treated endothelium. Besides, we demonstrated that, among the cytokines studied, the critical role in the process of atherogenesis is played by IL-1β and TNF-α. While, in the case of with MCP-1, RANTES and IL-10, the level of adhesion to the endothelium increased with their increasing concentrations in ATCM, their selective inhibition did not document any major effect on the monocyte adhesion. Moreover, ATCM was shown to provoke pro-inflammatory changes in gene expression, as documented by increased expression of adhesion molecule (ICAM-1, VCAM-1) as well as pro-inflammatory IL-6 genes and decreased expression of the anti-inflammatory TGF-β gene.

Using flow cytometry, we were able to define the phenotypes of polarized macrophages based on the surface markers in adipose tissue and the influence of individual cardiovascular risk factors on the proportions of polarized pro-inflammatory (M1) and anti-inflammatory (M2) macrophages. We demonstrated a relationship between increasing concentrations of non-HDL cholesterol and the proportion of M1 polarized macrophages, with VAT exhibiting a closer association compared with SAT. Obesity was associated with a higher proportion of pro-inflammatory macrophages only in SAT. Regarding VAT, the proportion of pro-inflammatory macrophage populations was higher in men, in those aged >51 years and, also, in individuals with hypercholesterolemia. By contrast, statin therapy was associated with a decrease in the proportion of macrophage populations in VAT. In conclusion, we have demonstrated a relation between the proportion of pro-inflammatory macrophages in adipose tissue and the major risk factors of atherosclerosis.