Obesity, hyperlipidemia, hypertension, insulin resistance, diabetes, and metabolic syndrome can cause chronic inflammation and metabolic disarray, and all are risk factors of atherosclerosis [1-7]. In mammals, adipose tissue (body fat) consists of two main types, brown and white. Brown fat generates body heat in newborns and in hibernating animals, while the principle purpose of white fat in healthy animals is energy storage. White adipose can be further divided into subcutaneous adipose, which is responsible for weight gain [8], while visceral adipose tissue is the last of the energy reserves and secretes a variety of hormones and cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), resistin, and adiponectin. For this reason, adipocytes have recently become recognized as endocrine cells [9]. Under disease conditions such as obesity, atherosclerosis, insulin resistance, diabetes, and others, adipocytes begin forming endocrine disorders leading to endocrine, autocrine, and paracrine dysfunction [10-12].

Secretion of Adipocytes and Macrophages under Conditions of Inflammation and/or Insulin Resistance and Effect of Adipocytes on Preadipocytes under These Conditions

Yu-Tao Wei1,2,3, Dong-Sheng Xia3,4, Wen-Kai Yang3,5, Xian-Guo Wang3, Xin-Zhong Chen3*, and Nian-Guo Dong3

1Department of Thoracic and Cardiovascular Surgery, Second Hospital Affiliated with the Medical College of Shihezi University (Department of Thoracic and Cardiovascular Surgery, Hospital of Xinjiang Production and Construction Corps), Wulumuqi 830002, Xinjiang, China
2Department of Thoracic and Cardiovascular Surgery, First Hospital Affiliated with the Medical College of Shihezi University, Shihezi 832008, Xinjiang, China
3Department of Cardiovascular Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei, China; E-mail: cxz1202065@163.com; wytyh@163.com
4Department of Cardiovascular Surgery, Henan Provincial People’s Hospital, Zhengzhou 450000, Henan, China
5Department of Cardiac Surgery, Jiangxi Provincial People’s Hospital, Nanchang 330006, Jiangxi, China

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Abstract—The purpose of the present study was to examine changes in preadipocytes following the coculture of preadipocytes and adipocytes and the effects on the secretion of adipocytes and macrophages following induction of inflammation and insulin resistance. Mature adipocytes and RAW264.7 macrophages were treated with lipopolysaccharide and insulin to establish models of inflammation and insulin resistance, respectively. The mRNA expression levels of IL-6, MCP-1, and TNF-α in all adipocyte treatment groups were significantly greater compared with the control, and that of adiponectin was less (P < 0.05). In the RAW264.7 macrophages, the mRNA expression levels of IL-6 and TNF-α were greater than those in the control group (P < 0.05). Moreover, the results of this study confirmed that adipocytes and macrophages increased the secretion of inflammatory factors under conditions of induced inflammation and insulin resistance. In addition, 3T3-L1 adipocytes inhibited the proliferation and differentiation of preadipocytes when cocultured with adipocytes under conditions of inflammation and/or insulin resistance, and the phenotype of preadipocytes did not change.

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Abbreviations: C/EBP, CCAAT-enhancer-binding protein; DAPI, 4′,6-diamidino-2-phenylindole; IBMX, 3-isobutyl-1-methylxanthine; IL-6, interleukin-6; IR, insulin resistance; MCP-1, monocyte chemotactic protein-1; MTT, methyl thiazolyl tetrazolium; PBS, phosphate-buffered saline; PID, post-induction day; PPARγ, peroxisome proliferator-activated receptor gamma; Pref-1, preadipocyte differentiation factor 1; RT-qPCR, real-time quantitative polymerase chain reaction; TNF-α, tumor necrosis factor-alpha.

* To whom correspondence should be addressed.
MATERIALS AND METHODS

Experimental materials, essential drugs, and reagents. The murine preadipocyte (3T3-L1, GNM25) and macrophage (RAW264.7, TCM13) cell lines were purchased from the Shanghai Chinese Academy of Sciences Cell Bank. The cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS), 100 μg/ml penicillin, and streptomycin at 37°C in a 5% CO2 saturated incubator. DMEM (12800017), FBS (10099141), and 0.25% trypsin (25200056) were purchased from Gibco (USA). Lipopolysaccharide, (L2880), insulin (1342106), dexamethasone (D4902), 3-isobutyl-1-methylxanthine (IBMX, I7018), 6-diamidino-2-phenylindole (DAPI, D9542), penicillin (P3032), streptomycin (S9137), and the dye oil red O (O1391) were from Sigma (USA); and 3T3-L1 preadipocytes to CD68, ab955, (rat monoclonal antibodies (KP1) to CD68, ab955, D9542), penicillin (P3032), streptomycin (S9137), and 0.25% trypsin (25200056) were purchased from Gibco (USA).

Recovery and culture with 3T3-L1 preadipocytes and RAW264.7 macrophage cells. The 3T3-L1 preadipocytes were seeded in 6-well plates at a density of 8000 cells/cm² and cultured according to standard conditions. Briefly, the cells were grown in 5% CO2 in DMEM containing 10% FBS, 2 mM L-glutamine, and a 1% penicillin–streptomycin mixture. Two days after the cells reached confluency, the cells were induced to differentiate in high-glucose DMEM containing 10% FBS, 10 mg/liter insulin, 0.5 mM IBMX, and 1 μM dexamethasone for 2 days. Fresh medium containing only insulin was changed every 2 days. Post-induction at day 0 (PID 0) refers to preadipocytes prior to induction. When 90% of the adipocytes presented the phenotype following PID 8, all analyses were conducted. RAW264.7 macrophage cells were seeded onto 6-well plates at a density of 8000 cells/cm² and cultured according to standard conditions.

Establishing inflammation and insulin resistance models in 3T3-L1 adipocytes. Prior to the assays, 3T3-L1 adipocytes were washed and maintained overnight in DMEM without FBS. Assays were conducted both under basal conditions and after stimulation with lipopolysaccharide (1 μg/ml, 18 h) [21] and/or insulin (1.7 μM, 18 h) [22] on differentiated cells. The concentrations and incubation times used were similar to those used in other experimental models [21, 22].

Cell treatment groups. Four treatment groups of 3T3-L1 adipocytes were created: A-1, the negative control (treated with an equivalent amount of phosphate-buffered saline, PBS); A-2, (insulin-resistance, treated with insulin, 1.7 μM, 18 h); A-3, inflammation (treated with lipopolysaccharide, 1 μg/ml, 18 h); and A-4, inflammation + insulin resistance (treated with lipopolysaccharide, 1 μg/ml, 18 h + insulin, 1.7 μM, 18 h). Four treatment groups of RAW264.7 macrophages were created using the same culture conditions and cell treatments as for the adipocytes as follows: B-1, negative control (treated with an equivalent amount of PBS); B-2, insulin-resistance; B-3, inflammation; B-4, inflammation + insulin resistance. Four treatment groups were also created with RAW264.7 macrophages and 3T3-L1 preadipocytes cocultured in transwell chambers. The RAW264.7 macrophages in the upper chamber and 3T3-L1 preadipocytes in the lower chamber were divided into C-1 to C-4 groups and were cultured for 48 h. The C-1 to C-4 groups in the lower chamber underwent routine testing and induction of differentiation. (Both RAW264.7 macrophages and 3T3-L1 preadipocytes were detected at the same order of magnitude.)

Real-time quantitative polymerase chain reaction (RT-qPCR) detection of gene expression. Total RNA was extracted from the RAW264.7 macrophages and 3T3-L1 preadipocytes stored in liquid nitrogen using TRIzol reagent (Invitrogen, USA) following the manufacturer’s instructions. Reverse transcription reactions were conducted using a Transcriptor First Strand cDNA Synthesis Kit (Roche, USA). The oligonucleotide primer sequences were designed by Premier Primer 5.0 software as shown in Table 1, and β-actin was used as the internal control. The synthesized first-strand cDNA samples were subjected to RT-qPCR with SYBR Green PCR Master Mix (Toyobo Bio-Technology, China), and PCR was performed using an ABI Prism 7700 Sequence Detector (Applied Biosystems, Japan). The abundance of each gene product was calculated using regression analyses against a standard curve generated by 2-fold serial dilutions of positive PCR controls for each gene. The