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Allergen exposure induces adipose tissue inflammation and insulin resistance

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ABSTRACT

This study investigates whether exposure to allergen elicits insulin resistance as a result of adipose tissue inflammation. Male C57BL/6 mice were challenged with ovalbumin (OVA) allergen for 12 weeks, and blood and adipose tissue samples were collected at 24 h after the last challenge. Levels of adhesion molecules, fasting insulin, fasting glucose, and adipokines in the blood were analyzed, and fasting homeostasis model assessment was applied to determine insulin resistance (HOMA-IR). The expression of pro- and anti-inflammatory genes in dissected adipose tissues was analyzed by real-time RT-PCR. Our results showed that OVA exposure increased insulin resistance as well as resistin and E-selectin, but reduced adiponectin in the serum. Resistin level was significantly correlated with HOMA-IR. Moreover, in adipose tissues of OVA-challenged mice, the pro-inflammatory M1 genes were more abundant while the anti-inflammatory M2 genes were less than those of PBS-treated mice. The expressional changes of both M1 and M2 genes were significantly associated with serum levels of adiponectin, resistin, and E-selectin. Hematoxylin and eosin (HE) and immunohistochemistry (IHC) stain also showed that there was more obvious inflammation in OVA-challenged mice. In conclusion, the current study suggests the relationship between allergen-elicited adipose tissue inflammation and circulating inflammatory molecules, which are possible mediators for the development of insulin resistance. Therefore, we propose that allergen exposure might be one risk factor for insulin resistance.

1. Introduction

Diabetes is a group of metabolic diseases that are prevalent in all countries, and the number of adults with diabetes is estimated to increase by 20% in developed countries and 69% in developing countries from 2010 to 2030 [1]. Diabetes is generally classified into type 1 and type 2, which are characterized by insulin deficiency and insulin resistance, respectively. As opposed to insulin deficiency that is partly inherited, the development of insulin resistance is primarily due to lifestyle factors such as diet and environmental pollutant exposure [2–5]. Understanding the risk factors for insulin resistance is crucial to the prevention and development of therapeutic strategies for type 2 diabetes.

Pathophysiological studies indicated that inflammation plays a key role in the development of insulin resistance [6,7]. For example, high-fat-fed mice produced more pro-inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1β (IL-1β) in the liver, which caused hepatic and systemic insulin resistance [8,9]. Inflammatory markers vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and endothelial-selectin (E-selectin), which are released from stimulated vascular endothelium, appear to participate in the development of insulin resistance [6,7,10,11]. Moreover, the levels of circulating VCAM-1, ICAM-1 and E-selectin were shown to significantly correlate with insulin sensitivity [12–14]. Therefore, inflammation is now known as a pathological condition that associates with the development of insulin resistance.

Adipose tissue produces a large number of bioactive mediators and has a central role in both immune responses and metabolism of lipid and glucose. Adipose tissue inflammation induced elevated plasminogen activator inhibitor-1 (PAI-1), IL-1β, IL-6, and TNF-α while it decreased the expression of anti-inflammatory markers like macrophage galactose lectin-1 (Mgl1), macrophage galactose lectin-2 (Mgl2) and interleukin-10 (IL-10), and alteration of these inflammatory factors

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appeared to promote insulin resistance [6,7,15–17]. Members of adipokines participated in glucose production and insulin resistance; in particular, adiponectin had a protective role in insulin resistance by increasing glucose uptake [18–20], while resistin was implied to increase the propensity of insulin resistance [21,22]. More importantly, epidemiological studies found that type 2 diabetes patients had higher serum resistin levels compared to healthy subjects [23,24], while several cohort studies indicated that a lower level of adiponectin is associated with a higher incidence of diabetes [25–27]. Taken together, the inflammatory factors and adipokines that are produced by adipose tissues may play an important role in the development of insulin resistance and type 2 diabetes.

We recently discovered that acute exposure to allergen lowered adiponectin levels in serum and adipose tissues and concurrently induced adipose tissue inflammation [28]. In the current study, we hypothesize that allergen exposure promotes insulin resistance through the action of adipose tissue inflammation. Our data of a long-term exposure model found that allergen could modulate serum IL-6, adipokines and E-selectin, and alteration of these circulating factors correlated with pro-inflammatory phenotype of macrophage in adipose tissues. More importantly, allergen-induced fluctuation of serum resistin is associated with insulin sensitivity that was obtained by homeostasis model assessment insulin resistance (HOMA-IR). Collectively, our study suggests that allergen exposure may be one risk factor for the development of insulin resistance.

2. Materials and methods

2.1. Animal

Eight-week-old male C57BL/6 mice were obtained from the National Cheng-Kung University Laboratory Animal Center. Mice were grouped and kept in constant lighting cycle (12-h light/dark), temperature (23 ± 2 °C) and relative humidity (50–60%) with free access to food and water. In this study, at least repeated experiments were performed until trend of the response curve was consistent before we started collection of study data. All study protocols were approved by the Animal Care and Use Committee of the National Cheng-Kung University.

2.2. Allergen sensitization and challenge

In the first stage, mice were sensitized with four intraperitoneal injections of 10 μg ovalbumin (OVA, Grade IV, Sigma-Aldrich, St Louis, MO, USA) bound to 1 mg aluminum hydroxide (Sigma-Aldrich, St. Louis, MO, USA) in 200 μl phosphate buffered saline (PBS, pH 7.4) on days 1, 8, 15 and 22. On day 29, for the second stage, the mice were intranasally challenged with 50 μg OVA in 25 μl PBS once a day and five days per week for 12 weeks. Equal volume of PBS was used in parallel experiments as a control. Twenty-four hours after the last intranasal challenge, mice were weighed (AE240, Mettler-Toledo, Switzerland) and sacrificed with cytosol (Kyorin Pharmaceuticals, Tokyo, Japan), and the blood, bronchoalveolar lavage fluid (BALF) and adipose tissue were collected.

2.3. Blood glucose, insulin, adiponectin, resistin and immune markers

Oral glucose tolerance test (OGTT) was performed at the eighth week of allergen challenge. Mice were deprived of food beginning at 9 AM for 4 h and then supplied with 400 mg/ml glucose (D-(+)glucose, Sigma-Aldrich, St. Louis, MO, USA) by gavage feeding. Blood samples were collected before and at 15, 30, 60 and 120 min after the feeding, centrifuged at 1250 ×g for 15 min at 4 °C, and stored at −80 °C. The blood glucose and insulin were determined by GLUC-PAP (RANDOX, Crumlin, United Kingdom) and insulin kit (Mercodia, Uppsala, Sweden) in accordance with the manufacturer's protocols, respectively.

Mice were sacrificed at 24 h after the last intranasal challenge with 4-hour fast. Blood samples were centrifuged at 12,500 rpm for 30 min at 4 °C, and the serum was stored at −80 °C. Enzyme-linked immunosorbent assays (ELISA) were performed to determine the serum levels of IL-6, TNF-α, adiponectin, resistin, VCAM-1, ICAM-1, E-selectin (R&D System, Minneapolis, MN, USA), and total IgE (BD Biosciences, San Diego, CA, USA). Fasting homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by fasting blood glucose (mg/dl) × fasting insulin (μU/ml) / 405 [29].

2.4. Cell populations in the bronchoalveolar lavage fluid (BALF)

This study has drawn BALF samples and determined the number and population of cell to understand whether allergen exposure successfully elicit airway inflammation. Two tubes of BALF were collected by order, and each included 3 repeated, 30-second-long drawings using 1 ml saline. The BALF was centrifuged at 4 °C and 2000 rpm for 10 min, and the pellets were re-suspended in 1 ml of RPMI 1640 medium supplemented with 10% fetal bovine serum. Twenty microliters of cell suspension was drawn for total cell counts by using a hemocytometer (Marienfeld, Germany) and a microscope (BH-2, Olympus, Tokyo, Japan). The rest of the suspension was cytocentrifuged (Thermo Electron Corporation, Waltham, MA, USA) to transfer cells onto a microscopic slide, which was subsequently stained with Diff-quik (Sysmex Corporation, Tokyo, Japan). Macrophages, eosinophils and neutrophils were differentiated by stain, and cell numbers of each population were counted under a microscope (CX31RBSF, Olympus, Tokyo, Japan).

2.5. Inflammatory marker expression in adipose tissues

Brown (interscapular) and white (inguinal and gonado) adipose tissues were harvested and conserved in RNAlater (Protech, Taipei, Taiwan) at 4 °C for 24 h before storage in −20 °C. For RNA extraction, 1 ml RNA Plus Extraction Reagent (Invitrogen, Carlsbad, CA, USA) was mixed with the adipose tissue, and the mixture was homogenized by a BeadBeater (BioSpec Products, Inc, Bartlesville, OK, USA). The extracted sample was further treated with RNA pure kit (Geneaid, New Taipei, Taiwan) to increase the purity. The mRNA expressions of PAI-1, IL-6, TNF-α, IL-1β (pro-inflammatory M1 macrophage), IL-10, Mgl1, and Mgl2 (anti-inflammatory M2 macrophage) in adipose tissues were determined by real-time quantitative RT-PCR. Reverse transcription was performed by using SuperScript III First-Strand Synthesis Super-Mix (Invitrogen, Carlsbad, CA, USA). Quantitative real-time RT-PCR was performed with Fast SYBR Green Master Mix (Applied Biosystem, Foster, California, USA) and SYBR Green system (Applied Biosystem, StepOne Plus Real-Time PCR SYSTEM Thermal CyclInguinal Block, Model 4376592, Foster, CA, USA). The data were expressed as relative gene expression to the endogenous reference, 18S rRNA, by the ΔΔCt method [30]. For comparison between the OVA- and PBS-treated groups, the level of hypoxanthine guanine phosphoribosyl transferase (HPRT) expression was used as the internal control. Primer sets were as follows: PAI-1 forward 5′-TGA TGG CTC AGA GCA ACA AG-3′ and reverse 5′-GCC AGG GTT GCA CTA AAC AT-3′; IL-6 forward 5′-GAT GCT ACC AAA CTG GAT ATA ATC-3′ and reverse 5′-GGT CCT TAG CCA CTC CTT CTG TG-3′; TNF-α forward 5′-ATG AGC ACA GAA AGC ATG-3′ and reverse 5′-TCA CAG AGC AAT GAC TCC-3′; IL-1β forward 5′-GCA GCT ATG GCA ACT GTT CCT-3′ and reverse 5′-TCA TAT GGG TCC GAC AGC ACG-3′; IL-10 forward 5′-CAG AGC CAC ATG CTC CTA GA-3′ and reverse 5′-TGT CCA GCT GGT CCT TTG TT-3′; Mgl1 forward 5′-TGA GAA AGG CTT TAA GAA CTG GG-3′ and reverse 5′-GAC CAC CTG TAG TGA TGT GGG-3′; Mgl2 forward 5′-TTA GCC AAT GTG CTT AGC TGG-3′ and reverse 5′-GGC CTC CAA TTC TTG AAA CCT-3′; and HPRT forward 5′-CTC ATG GAC TGA TTA TGG ACA GGA-3′ and reverse 5′-GCA GGT CAG CAA AGA ACT TAT AGC-3′.