Polarized th2 cells attenuates high-fat-diet induced obesity through the suppression of lipogenesis

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Research Article

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Abstract

Immune cells such as macrophages, B cells, neutrophils and T cell subsets are involved in obesity. However, the tissue-specific function of Th2 cells in adipose tissue remains unknown. We show here that adoptive transfer of polarized Th2 cells to CD3ε knockout (CD3ε−/−) mice attenuates obesity after high-fat-diet (HFD), accompanied by relieved hepatic steatosis, glucose intolerance, and insulin resistance. Mechanistically, Th2 cell treatment promoted oxidative phosphorylation of adipocytes, contributing to the decrease of lipid droplet accumulation. Our data indicate that Th2 cell treatment is a novel approach to the treatment of diet-induced obesity and other diseases involving lipid droplet accumulation disorders.

Introduction

Obesity has become a worldwide health problem for which some related diseases may occur such as type 2 diabetes[1], fatty liver disease[2], atherosclerosis[3], cardiovascular problems, as well as cancer[4] and autoimmune diseases[5]. Obesity is affected by many factors such as diet, exercise, genetics, environment, stress, emotion and poor sleep. It is worth noting that high-fat diet has become the main cause of obesity, especially in western countries. White adipose tissue has been recognized as the main site of energy balance through the regulation of both food intake and energy expenditure [6]. White adipocytes, the predominant cell type in white adipose tissue, store dietary energy in a highly concentrated form as triglyceride, mostly in a single large lipid droplet. The number of adipocytes remains constant after adulthood, while its size can become larger with the increase of intracellular triglyceride storage. The content of lipid droplets in adipocytes is an important factor in determining obesity in white adipose tissue. Therefore, an important approach to address the problem of obesity is to target adipocytes.

In addition, obesity is now recognized as a chronic low-grade inflammatory disease and is associated with the chronic inflammation within adipose tissue. Typically, in acute inflammation the initial damaging insult triggers the release of a number of immunomodulatory molecules including cytokines and chemokines from tissue resident macrophages and mast cells provoking a rapid recruitment of firstly neutrophils, then macrophages and lymphocytes from circulation to the inflammation site[7]. Recent study suggests that adipocytes and macrophages employ intercellular mitochondria transfer as a mechanism of immunometabolic crosstalk that regulates metabolic homeostasis and is impaired in obesity[8]. Furthermore, dendritic cells[9], mast cells[10], neutrophils[11], eosinophils[12]. In addition, T cell subsets (CD8+ T cells[13], th17[14], th1[15], Tregs[16], NKT[17]) also participates in high-fat diet induced obesity and related metabolic disorders. Obesity aggravated the immune histopathological characteristics in the eosinophilic esophagitis experimental model, which was associated with the reduction of the regulatory profile, and the increased inflammatory cells influx, related to the th2 profile[18].

In this study, we report the effects of polarized Th2 cells on HFD mice. We further show that Th2 cells can alleviate fat accumulation of adipose tissue and steatosis in mice fed on HFD. Moreover, Th2 treatment
suppresses mitochondrial dysfunction both in vitro and in vivo in the context of intestinal epithelial damage. The beneficial effects of mannose rely on its protection against lysosomal rupture and cathepsin B release in damaged intestinal epithelial cells. Our data suggest the potential use of mannose supplementation to treat IBD and other diseases related to tight junction dysfunction and lysosomal disorders.

Materials And Methods

Mice and treatments

Male LATY136F mutant C57BL/6J mice and CD3ε knockout C57BL/6J mice were obtained from Centre d'Immunologie de Marseille-Luminy in France. Mice were maintained under SPF conditions. All animal experiments in this study were approved by the Welfare and Ethical Committee for Experimental Animal Care of Xinxiang Medical University. These mice under standard laboratory conditions with controlled temperature (19–22 °C) and 12-h light/dark cycle. Purified Th2 cells were obtained from mesenteric lymph nodes of LATY136F mutant C57BL/6J mice with CD5 MicroBeads (130-049-301 Miltenyi) following the standard protocol. 2 x 10^6 purified Th2 cells in 200 μl PBS or 200 μl PBS only were injected into CD3ε knockout C57BL/6J mice via tail vein respectively. After that, the two group mice were subjected to high-fat diet (D12492i, Research Diets, 60 kcal%) for 16 weeks.

Metabolic and physical activity measurements

Animals were placed individually in chambers for 5 consecutive days at ambient temperature with 12 h light/dark cycles. Animals had free access to food and water. At the end of 16 weeks, the mice were given 2 days of acclimation in metabolic chambers before the trial and then continuously recorded for 96 h with the following measurements being taken every 30 min: food intake, water intake, ambulatory activity (in X and Z axes), and gas exchange (O2 and CO2). All measurements were taken automatically through the use of the SABLE SYSTEMS INERNATIONAL (USA).

Glucose and insulin tolerance test

The glucose tolerance test (GTT), and insulin tolerance test (ITT) were conducted in mice after 6-h fasting. The mice were intraperitoneally injected with 2 g/kg glucose for GTT, and 0.75 U/kg insulin for ITT. In the studies, the blood glucose was tested at six time points (0, 15, 30, 60, 90 and 120 minutes).

RNA isolation and real time RT-PCR

Total RNA was isolated from animal tissues with TRIlzol (Invitrogen, Carlsbad, CA). RNA concentration was measured by a spectrophotometer (Thermo Scientific, Ltd, Waltadult mouse, MA). The first strand of cDNA was obtained using the PrimeaScript™ II 1st Strand cDNA synthesis kit (Takara, Ltd, Japan) according to the manufacturer's instruction. Subsequently, SYBR green-based qPCR was performed using a power SYBR greenmaster mix (Applied Biosystems, Foster City, CA) and 7500 Fast Real-Time PCR
system (Applied Biosystems, Foster City, CA). The expression was normalized to mouse ribosome 18S rRNA.

**Western blots**

EAT and liver were collected from mice and snap frozen in liquid nitrogen and splitted in RIPA buffer of protease inhibitors. Homogenates were cleared by centrifugation (10000 rpm for 30 min at 4°C). Protein lysates (30 μg) were separated on 10% SDS-PAGE and transferred on polyvinylidifluoride membranes (Millipore, Bedford, USA). Membrane were blocked in tris-buffered saline (TBS, pH 7.4) containing 5% nonfat milk at room temperature for 1 h and incubated with the primary antibodies overnight at 4°C. After washing, the membranes were incubated with the HRP-conjugated secondary antibodies at room temperature for 1 h. Detection and analysis was performed with Chemidoc XRS Image system and Image Lab 5.0 software (Biorad).

**Cell culture and induction of adipocyte differentiation**

3T3-L1 cells were maintained in DMEM supplemented with 10% calf serum and 5% CO2. For adipocyte differentiation, the cells were differentiated with 10 μg/mL insulin, 1 μM dexamethasone and 0.5 mM 1-methyl-3-isobutylxanthine (IBMX) in DMEM containing 10% (v/v) fetal bovine serum (FBS) and 1% penicillin-streptomycin for 2 days. The medium was then replaced by a medium containing only insulin. After 2 days, this maintenance medium was discarded. Differentiated adipocytes were cultured in DMEM containing 10% FBS.

**Histology**

Mice were killed at indicated time points and adipose tissue and liver were carefully harvested. For hematoxylin and eosin (H&E) staining, tissues of mice were fixed in 4% paraformaldehyde (PFA) overnight at 4°C, followed by dehydration in 70% ethanol. After the dehydration procedure, tissues were embedded in paraffin, sectioned at a thickness of 5mm, and stained with H&E following the standard protocol. Images were acquired using a microscope (LEICA 500).

**Co-culture of Th2 Cells and 3T3-L1 Preadipocytes**

3T3-L1 preadipocyte cells were plated in 24 wells at a density of 1×10⁵ cells/well and maintained in DMEM supplemented with 1% penicillin/streptomycin and 10% FBS. To evaluate the effect of Th2 cells on 3T3-L1 induction and differentiation, 2-days-postconfluent cells (day 0) were treated with different number of purified Th2 cells (5×10⁴ and 1×10⁵ Th2 cells) and induction medium (DMEM contains10% FBS, 1.73μM insulin, 1μM dexamethasone, 0.5mM 3 -isobutyl-1-methylxanthine, 0.2μM indomethaein and 1μM pioglitazone) every 2 days until day4, and control group was treated without Th2 cells. The medium was replaced with induction medium (DMEM contains10% FBS and 1.73μM insulin) every 2 days until day 8.

**Statistical analysis**
All results were expressed as mean ± SEM. Statistical significance between two groups was evaluated using the Student’s t-test, while comparisons of multiple groups were assessed by two-way analysis of variance (ANOVA), followed by Student-Newman-Keul's test. p<0.05 was considered significant. For morphometric analyses, quantification of micrographs was independently reviewed by two observers and the average of their scoring was used for each micrograph. Quantification was performed in images after appropriate thresholding using the Image J software (NIH Image).

Results

1. Polarized Th2 cells protect against HFD induced obesity of mice.

To determine the function of Th2 cells in obesity, we first evaluated whether lipid accumulation in adipose tissue could be changed by Th2 cells adoptive transfer in the condition of HFD. Eight-week-old CD3ε−/− male mice were randomly divided into two groups: 60% HFD with injection of PBS and 60% HFD with intravenous injection of Th2 cells isolated from LAT^{Y136} mice. Mice transferred with Th2 cells were resistant to weight gain after 16 weeks HFD (Fig. 1A). The recipient mice were noticeably leaner than control group after 16 weeks of HFD treatment (Fig. 1B). The epididymal adipose tissue (EAT), subcutaneous adipose tissue (SAT), perirenal adipose tissue (PRAT) and brown adipose tissue (BAT) were markedly reduced in the recipient mice, consistent with a notable decrease in the size of adipocytes (Fig. 1C, D, E). Western blot analysis further confirmed the reduced protein level of ACSL (long-chain acyl-CoA synthetase), FAS (fatty acid synthase), Acetyl-CoA Carboxylase (ACC) and lipid droplets marker perilipin-2 in Th2 cells transfer group compared to the control group (Fig. 1F). In addition, the biochemical analysis revealed that Th2 cells also reduced serum triglyceride (TG), cholesterol (TC) and high-density lipoprotein (HDL) concentrations and increased serum low-density lipoprotein (LDL) of CD3ε−/− mice fed the HFD (Fig. 1G).

2. Th2 cells prevent HFD-induced hepatic steatosis.

With westernization of dietary habits increased, the prevalence of non-alcoholic fatty liver disease (NAFLD) has reached 25% of the world population[19]. To determine the role of Th2 cells in fatty liver, representative photograph of liver (Fig. 2A) and liver index (Fig. 2B) showed that increased liver weight in Th2 cells-transferred mice compared to control mice. However, Th2 cells adoptive transfer substantially attenuated the lipid accumulation of liver upon HFD according to the H&E (Fig. 2C) and oil red staining (Fig. 2D). Consistently, Th2 cells transfer also decreased both triglycerides (Fig. 2E) and cholesterol (Fig. 2F) levels of liver. The mRNA and protein expression of lipid synthesis decreased in the liver of recipient mice fed on HFD (Fig. 2G, H).

3. Th2 cells reverse HFD-induced metabolic imbalance.

To explore the potential role of Th2 cells in regulating food intake and whole-body energy homeostasis during HFD, mice were monitored for energy expenditure and food intake. Exposure to HFD for 16 weeks had no significant differences in food intake which was corroborated by using in-depth analysis with an
automated food intake-monitoring system (Fig. 3A). In accordance with the increased body weight gain and elevated body fat composition, a significant impairment in whole-body energy expenditure including O2 consumption and CO2 production were observed in the CD3KO mice but not in the Th2 cells-transferred CD3ε−/− mice fed on HFD (Fig. 3B-E). Similarly, Th2 cells can also increase heat production under HFD condition (Fig. 3F, G). These results suggest that Th2 cells transfer drastically restore whole-body energy expenditure during HFD.

4. Th2 cells improve glucose homeostasis and insulin sensitivity.

To explore the effects of Th2 cells in metabolic related parameters, fasting glucose and insulin levels of mice plasma after 6 h fasting. Th2 cells transfer resulted in significantly decrease of both glucose and insulin concentration (Fig. 4A, C). Th2 cells-transferred mice also showed better performance in both the glucose tolerance test (GTT) and insulin tolerance test (ITT) (Fig. 4B, D). In addition, adiponectin and leptin level of Th2 cells-transferred mice were lower compared to the control mice upon HFD treatment (Fig. 4E). Western blot analysis further confirmed the increased protein level of phospho-AKT in Th2 cells transfer group compared to the control group (Fig. 4F). Taken together, these data indicate that Th2 improves glucose tolerance and insulin sensitivity.

5. Th2 treatment CD3KO mice show Th2 type inflammation.

Due to inflammation is closely related to glucose intolerance and insulin resistance, we explored systemic and local inflammation in mice in the presence or absence of Th2 cells transfer. Pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin 1-β (IL-1β) and interferon-γ (IFN-γ) have no difference in serum and EAT of Th2 cells-transferred mice and the control mice (Fig. 5A, C). We also examined and compared the levels of Th2 type cytokines between CD3ε−/− and Th2 cells-transferred CD3ε−/− mice. The result showed that decreased metabolic disorder in recipient mice was accompanied by a significant elevation of Th2 type cytokines (i.e., IL-4, IL-5, IL-6, IL-10 and IL-13) (Fig. 5B, D). In summary, Th2 cells increases the expression of Th2 type cytokines in serum and EAT of mice, which may be one of the important factors leading to the alleviation of metabolic disorders in mice upon HFD conditions.

6. Th2 cells inhibit adipocyte differentiation.

The excessive accumulation of lipid droplets in adipocytes is the direct cause of obesity, so it is speculated that Th2 cells may be involved in the differentiation of adipocytes. The results show that Th2 can inhibit the differentiation of adipocytes (Fig. 6A, B). The results of qRT-PCR and western blot showed that 7 days after adipogenic differentiation, the expression levels of transcription factors related to adipocyte differentiation were significantly lower in the Th2 cells treatment group compared with the NC group (Fig. 6C, D).

7. Th2 cells reduce the accumulation of lipid droplets in adipocyte through mitochondrial oxphos.
The metabolism of adipocytes affects the accumulation of lipid droplets in adipocytes, so we tested the effect of Th2 cells on the level of adipocyte oxidative phosphorylation (oxphos). The results show that Th2 cells treatment promotes the expression of mitochondrial aerobic respiration related enzymes (oxphos and PDH) (Fig. 7A). To further validate the role of oxphos in Th2 cells-mediated protection against obesity, adipocytes were pretreated with a specific oxphos inhibitor, rotenone, prior to Th2 cells treatment. After rotenone treatment, Th2 cells did not affect the accumulation of lipid droplets in fat cells (Fig. 7B, C, D).

**Discussion**

T cells, especially Th2 cells are involved in the chronic inflammation of adipose tissue in obese mice. Here, we report that the frequency of Th2 cells in adipose tissue was decreased upon HFD. We also identified a beneficial effect of Th2 cells in preventing obesity by modulating lipolysis. Molecular and cellular studies further revealed that Th2 cells facilitate mitochondrial energy metabolism in adipocytes by inhibiting fat synthesis and increasing the levels of oxidative phosphorylation.

The lipid accumulation of adipocytes plays crucial roles in the homeostasis of adipose tissue. Dysregulated lipid formation of adipose tissue has been implicated in the development of obesity. Our study shows that Th2 cells protect against obesity and nonalcoholic fatty liver induced by HFD. Indeed, Cytokines secreted by Th2 cells have been linked to the HFD induced obesity as well as the chronic inflammation of adipose tissue. Furthermore, our data show that upregulation of glucose tolerance and insulin sensitivity in the Th2 cells-transferred mice is involved in the AKT pathway.

Obesity is characteristic by low-grade, chronic inflammation. Various of immune cells such as macrophages, neutrophils, B cells and T cells involved in inflammation of adipose tissue. Macrophages make up to 40% of all AT cells in obese mice compared to 10% in lean mice. Our data also suggest that the increased accumulation of macrophages in adipose tissue of the obese is due to an influx of bone marrow–derived precursors into adipose tissue and their subsequent differentiation into mature F4/80-expressing macrophages[20]. Brotfain et al. validated that that superoxide production from unstimulated or stimulated neutrophils with the physiological stimuli and neutrophil migration were elevated in obese subjects while all other functions were normal, which indicates the capability of neutrophils to combat infections[21]. B cells are also critical regulators of inflammation in T2D due to their direct ability to promote pro-inflammatory T-cell function and secrete pro-inflammatory cytokines[22]. Moreover, T cell subsets including CD8 + T cells (CD8 + effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity), Th1 cells [23], and Tregs [24] have been reported to play an important role in diet-induced obesity inflammation. Here, we observed the Th2 cells-transferred CD3e−/− mice exhibited reduced fat accumulation accompanied by improvement of insulin sensitivity and glucose tolerance compared with CD3e−/− counterparts, indicating that Th2 cells limit the pathogenesis and progression of obesity. It is widely known that Th2 cells orchestrate protective type 2 immune responses, such as those that target helminths and facilitate tissue repair, but also contribute to chronic inflammatory diseases, such as asthma and allergy. Meanwhile, Th2 cells also perform a regulatory role in the immune system.
Therefore, we postulated that Th2 cells might be critical not only in immune regulation but also in metabolic diseases.

The effect of Th2 cells on the protection of obesity could also be manifested through regulating lipid accumulation of adipocytes. As our data suggested, Th2 cells have potent activity in globally decreasing the expression of specific adipocyte markers, as well as the transcription factor PPAR-γ, SREBP1, C/EBPα, and FABP4. It is noteworthy to mention that adipocyte differentiation contributes to the progressive metabolic disorders in patients with obesity. Herein, we provided the evidence for the first time that Th2 cells could inhibit adipocyte differentiation, which strongly expanded our understanding of the pathogenesis of obesity. Dysfunction of mitochondria results in detrimental effects on adipocyte differentiation, lipid metabolism, insulin sensitivity, oxidative capacity, and thermogenesis, which consequently lead to metabolic diseases [25]. To the best of our knowledge, our work reports for the first time that Th2 cells effectively regulate mitochondrial respiration in adipocytes.

Conclusion

In summary, our study demonstrates that Th2 cells attenuate obesity, fatty liver and metabolic dysfunction on a high-fat diet by reducing lipid accumulation of adipose tissue. Mechanistic studies revealed that Th2 cells inhibit adipocyte differentiation through increasing mitochondrial-mediated aerobic respiration of adipocytes.

Declarations

Ethics approval and consent to participate

Animal experiments in this assay had procured ethical approval from the Xinxiang medical University.

Consent for publication

Not applicable.

Availability of data and materials

The data and materials supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HW, LD, and JG designed research. LD, JG, LY, SL, YZ, WZ, and YL performed experiments. LD, JG, LY, and SL analyzed data. HW, LD, and JG wrote the manuscript. All authors reviewed the manuscript.

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Figures

Figure 1

Th2 cells reduce HFD induced obesity of mice.

Eight-week-old CD3ε−/− male mice were intravenous injection of Th2 cells isolated from LATY136 mice or PBS in the presence of HFD (60%) for 16 weeks respectively. (A) The body weight changes during the experiments were monitored. (n = 6). (B) Photographs of representative mice fed on HFD. Representative appearance of EAT, SAT, PRAT and BAT (C) and ratio of tissue weight to body weight (D). (E) Representative H&E staining of EAT, SAT, PRAT and BAT. Scale bars=50 µm. (F) The protein expression of ACSL1, FAS, ACC and ADRP was determined by western blot analysis. (G) The serum concentrations of
TG, TC, HDL and LDL were evaluated at the indicated time points. *$P<0.05$, **$P<0.01$, NS, not significant. Data from one representative experiment of three independent experiments are presented.

Figure 2

**Th2 cells relieve fatty liver.**

Livers were isolated on the last day of the experiment. A representative photograph of liver from each group is provided (A), and the liver weight was recorded (B). The histological analysis of mouse liver was performed by hematoxylin and eosin (H&E) (C), and oil red staining (D). Scale bar=100 μm. (E) Hepatic TG level. (F) Hepatic cholesterol level. (G) The mRNA levels of acetyl-CoA synthetase (AceCS1), ATP-Citrate Lyase (ACL), sterol regulatory element binding protein 1 (SREBP1c), fatty acid-binding protein 4 (FABP4), long-chain acyl-CoA synthetase 1 (ACSL1), peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding proteins (C/EBP) in livers were examined by qRT-PCR analysis. The related protein expression was examined by western blot analysis.
**Figure 3**

**Th2 cells improve metabolic parameter.**

Eight-week-old CD3ε−/− male mice were intravenous injection of Th2 cells isolated from LAT^{Y136} mice or PBS in the presence of HFD (60%) for 16 weeks respectively. (A) food intake, (B, C) O₂ consumption, (D, E) CO₂ production, and (F, G) heat production during both the light and dark cycles determined by CLAMS.
**Figure 4**

**Th2 cells remit glucose intolerance and insulin resistance.**

(A) Fasting plasma glucose levels, (B) insulin levels, (C) intraperitoneal glucose tolerance test plasma glucose concentrations, (D) insulin tolerance test, (E) plasma adiponectin and leptin were measured at 24 weeks of age. Areas under the curve were compared. Immunoblots of phospho-Ser473 Akt (p-Akt), and
Akt in the (F) eWAT and (G) liver of mice, the levels of p-Akt were normalized to Akt, respectively. (H) Representative H&E staining of pancreas. Values shown are mean±SD (n=6). *P<0.05 and **P<0.01.

Figure 5

Th2 cells cause Th2 type inflammation.

The mRNA levels of pro-inflammatory cytokine (TNF-α, IL-1β and IFN-γ) (A) and Th2-type cytokines (IL-4, IL-5, IL-6, IL-10, IL-13) (B) in eWAT were determined by qRT-PCR analysis. The serum level of TNF-α, IL-1β, IFN-γ, IL-4, IL-5, IL-6, IL-10 and IL-13 were examined by Luminex High Performance Assay.
**Figure 6**

**Th2 cells inhibit adipocyte differentiation.**

3T3-L1 preadipocyte cells were induced into adipocytes firstly and then co-cultured with different number of Th2 cells for 24 hours. (A) Morphology of fat cells were recorded in bright field. (B) Adipocytes differentiation was determined by staining with oil red with different numbers of Th2 cells. Scale bar = 50 μm. (C) The mRNA levels of PPAR-γ, SREBP1, C/EBPα, and FABP4 in adipocytes were determined by qRT-PCR analysis. (D) The protein level of AceCS1, ACSL1, FAS, p-ACC, and ADRP was evaluated by immunoblotting analysis.
**Figure 7**

**Th2 cells inhibit adipocyte differentiation through oxphos.**

(A) 3T3-L1 preadipocyte cells were induced into adipocytes firstly and then co-cultured with different number of Th2 cells for 24 hours, then the protein levels of PDH and OXPHOS were detected by immunoblotting. (B-D) 3T3-L1 preadipocyte cells were induced into adipocytes firstly and then co-cultured with different number of Th2 cells with rotenone for 24 hours. (B) The mRNA levels of PPAR-γ, SREBP1, C/EBPα, and FABP4 in adipocytes were determined by qRT-PCR analysis. (C) The protein level of AceCS1, ACSL1, FAS, p-ACC, and ADRP was evaluated by immunoblotting analysis. (D) Adipocytes were determined by staining with oil red. Scale bar = 50 μm.