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# Effects of Adenovirus-Mediated Overexpression of *JAZF1* on Chronic Inflammation: An *In Vitro* and *In Vivo* Study

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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**Background:** Insulin sensitivity and inflammation can be affected by juxtaposition with another zinc finger gene 1 (*JAZF1*), but its precise role in chronic inflammation is unclear. In this study, *JAZF1*-overexpression adenovirus plasmids were transfected into macrophages, $CD4^+$ T cells, and C57BL/6J mice to assess the role of *JAZF1* in chronic inflammation.

**Material/Methods:** *JAZF1* was cloned into an adenovirus skeleton plasmid and transfected in HEK293 cells to package and enrich the virus particles. *In vitro*, the *JAZF1* overexpression adenovirus vector (PAD-JAZF1) was cultured with peritoneal macrophages and peripheral blood $CD4^+$ T cells of C57BL/6J mice, and samples were evaluated using flow cytometry. *In vivo*, PAD-JAZF1 was introduced into C57BL/6J mice, and livers were collected to evaluate factors related to inflammation by hematoxylin & eosin and immunohistochemical staining.

**Results:** *In vitro*, PAD-JAZF1 decreased total macrophages, $CD11c^+$ macrophages, and the secretion of proinflammatory cytokines, but increased $CD206^+$ macrophages. It also decreased total $CD4^+$ T cells, active T cells, memory T cells, and the secretion of IL-6, IL-10, and IFN-γ, but increased Treg cells and restrictive T cells. *In vivo*, compared to those in the control group transfected with the adenovirus skeleton vector, mice transfected with the PAD-JAZF1 recombinant adenovirus had fewer $CD11c^+$ ATMs and $CD4^+$ T cells, lower levels of TNF-α and IL-6, and higher IL-10 concentrations in the liver.

**Conclusions:** These findings indicate that *JAZF1* limits chronic inflammation by reducing macrophage and $CD4^+$ T cell populations, altering subtype differentiation, and regulating the secretion of immune-related factors.

**MeSH Keywords:** Adenoviridae • Genes, vif • Macrophages • Natural Killer T-Cells

**Full-text PDF:** https://www.basic.medscimonit.com/abstract/index/idArt/924124







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## Background

The potential roles of insulin resistance and chronic inflammation in the pathogenesis of type 2 diabetes (T2D) have already demonstrated [1]. Inflammatory factors affect adipose tissues, oxidative stress, and the immune system, and then cause insulin resistance and β cell structure and function disorders, which play an important role in the progression of T2D. Preclinical evidence suggests that pancreatic β cell dysfunction is associated with the systemic inflammatory state, including C-reactive protein and its upstream regulator interleukin-6 (IL-6) [2]. A study conducted by Redwine et al. found that C-reactive protein and IL-6 are significantly correlated with both insulin sensitivity and β cell function [3]. Further, inflammation activates the internal insulin immune response. Chronic local inflammation of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) can result in the simultaneous secretion of a variety of fat-specific inflammatory factors involved in glycolipid metabolism [4]. Adipose tissue contains numerous immune cells, including T cells, B cells, and macrophages. $CD4^+$ T cells are involved in the initial stage of adipose tissue inflammation during the progression of impaired glucose tolerance and insulin resistance. This suggests that in adipose tissues of patients with T2D, $CD4^+$ T cells play important roles in the onset and maintenance of chronic inflammation [5].

Our preliminary studies showed that *JAZF1* can regulate antigen presentation by macrophages and thus the inflammatory state [6]. *JAZF1* (juxtaposed with other zinc finger gene 1) encodes a 27-kDa protein and includes 3 zinc finger motifs. Insulin sensitivity and insulin secretion are lower in the East Asian population than in European populations. In a comparison of candidate genes involved in susceptibility to T2D between the East and West, Xian et al. found that divergence in *JAZF1*, at 0.7%, was greatest among all genes [7]. This gene is highly expressed in adipose and liver tissues, which can affect the regulation of gluconeogenesis, insulin sensitivity, lipid metabolism, and inflammatory reactions [8]. There is also a correlation between *JAZF1* polymorphisms and the development of T2D [9]. However, the mechanism underlying this relationship is unclear. In this study, the effects of *JAZF1* on $CD4^+$ T cell and macrophage populations were investigated *in vitro* and *in vivo* to determine the precise roles of this locus in chronic inflammation.

## Material and Methods

### Ethics statement

All animal experiments were conducted according to the guidelines of the Ethics Committee of the Army Military Medical University.

### Flow cytometry and reagents

For the *in vitro* experiments, markers of inflammation were measured using the BD CBA Mouse Th1/Th2 Cytokine Kit (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Macrophages, $CD4^+$ T cells, and their subtypes were labeled with anti-mouse antibodies as $CD3^+$, $CD4^+$, $CD11b^+$, $CD11c^+$, $CD206^+$, $F4/80^+$, $CD25^+$, $CD44^+$, $CD69^+$, $CD152^+$, and Foxp3+ (BD Biosciences). Cells were treated with LPS (1 μg/mL, 50 μL; BD Biosciences) to induce differentiation. The FACSCalibur Flow Cytometer (BD Biosciences) and FCAP were used to analyze cells based on the manufacturer's instructions.

### Construction of adenovirus vectors to overexpress JAZF1

#### *Construction of the adenovirus shuttle plasmid*

A plasmid with JAZF1, pIRES2-JAZF1, was provided by our task group. The recombinant adenovirus used in this study was prepared using the AdEasy-$1^{XL}$ Adenoviral Vector System (Stratagene, La Jolla, CA, USA). JAZF1 was digested with XhoI and EcoRI from the plasmid pIRES2-JAZF1, ligated with Pshuttle-CMV, amplified in DH-5a, selected, purified using the Plasmid Maxiprep Kit (OMEGA, Irving, TX, USA), and identified by XhoI and EcoRI digestion and DNA sequencing; the resulting plasmid was named Pshuttle-JAZF1.

#### *Homologous recombination of the adenovirus skeleton plasmid (pAdEasy) and Pshuttle-JAZF1*

A shuttle plasmid carrying the target gene fragment *JAZF1* (Pshuttle-JAZF1) was linearized with PmeI and transferred into *Escherichia coli* BJ5183 with the adenovirus large skeleton plasmid (pAdEasy) for homologous recombination. The adenovirus skeleton plasmid was ampicillin-resistant; when it was recombined with the shuttle plasmid, ampicillin resistance was lost, and a kanamycin resistance gene was expressed. This change in resistance enabled selection of the recombinant adenovirus vector skeleton, which was confirmed by performing a DNA miniprep and PacI digestion. Plasmids from correct clones were amplified by transformation into XL10-Gold cells. Again, plasmid DNA was prepared by a standard procedure. The adenoviral DNA was verified by PacI digestion and DNA sequencing, followed by transformation into XL10-Gold cells for large-scale amplification. The correct recombinant was named pAD-JAZF1.

#### *Propagation, purification, titer determination, and identification of the pAD-JAZF1 adenovirus*

The recombinant adenovirus was propagated in human embryonic kidney 293 cells (HEK293) cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin,





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and 100 mg/mL streptomycin at 37°C with 5% $CO_2$. Twenty-four hours before transfection, 5 × $10^5$ cells were seeded on a 6-well plate until 80% confluence was reached. The recombinant adenovirus pAD-JAZF1 from correct clones was linearized with PacI and transfected into 293 packaging cells using liposomes (Hanbio). Due to the loss of the early gene E1 in the adenovirus vector genome, 293 cells with E1 were used as packaging cells. After the transfected cells were incubated continuously for 5–7 days, the cytopathic effect (CPE) was observed. Then, viruses were harvested and purified on CsCl gradients and titers were determined. They were subsequently stored at –80°C in 4% sucrose buffer.

*Determination of recombinant adenovirus titration*

The titer of recombinant adenoviral plasmids was measured by a plaque formation assay. AD293 cells were seeded (1×$10^6$ cells per well) in 6-well plates until they reached 50–70% confluence, which was followed by the addition of serial dilutions of viral samples. The cells were incubated in a 5% $CO_2$ incubator at 37°C for 10 days. Cell monolayers were fixed with 25% formaldehyde. Then, plaques were counted by staining with neutral red according to the following formula: number of plaques/dilution coefficient×volume of the viral solution.

**Cell culture, *in vitro* infection, and measurement of inflammatory mediators**

*Preparation of C57BL/6J mouse peritoneal macrophages*

Wild-type C57BL/6J mice at 8 weeks of age (22–28 g, normal chow diet) were used. DMEM with 5 mL of 75% serum was injected into the mouse abdomens. After 30 min, the liquid was recovered. Then, the mice were euthanized by cervical dislocation, placed in 75% alcohol for 3 min, injected with 5 mL of pre-cooled serum-free DMEM into the abdominal cavity, and washed, which was followed by collection of the liquid. After centrifugation at 200×*g* for 10 min, the upper liquid was discarded, and this was followed by washing with serum-free DMEM and the suspension of cells in DMEM-10% serum to obtain a concentration of approximately 2×$10^6$ cells/mL. The cell suspension was inoculated into a 96-well dish and incubated at 37°C with 5% $CO_2$. After 2 h, it was removed and cleaned, and preheated DMEM-10% serum was washed twice to obtain cells.

*Co-culture of peritoneal macrophages with recombinant adenovirus PAD-JAZF1*

Two groups were established, a PAD-JAZF1 group and a pAdEasy blank virus (control) group. The C57BL/6J mouse peritoneal macrophages prepared as described previously herein were added to 48-well plates for each group and 200 µL of preheated DMEM-10% serum medium was added for culture at 37°C with 5% $CO_2$. After 2 h, the recombinant adenovirus (5×$10^7$ PFU) and 50 µL of LPS (1 µg/mL) were added to each well. After 3 or 6 h, the cellular extract was collected and the macrophage subtypes ($CD11c^+$ and $CD206^+$) and inflammatory factors IL-1β, TNF-α (3 h), and IL-6 (6 h) were detected by flow cytometry.

*Selection and culture of $CD4^+$ T cells*

Peripheral blood mononuclear cells in C57BL/6J mice were isolated by the immunomagnetic bead method, and PE-$CD4^+$ T cells were separated by flow cytometry. The cell culture solution was IMDM containing 2 M glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, 50 µM β-mercaptoethanol, and 10% FBS. $CD4^+$ T cells were cultured in a cell culture dish containing anti-CD3 (5 µg/mL) and anti-CD28 (1 µg/mL) antibodies in a 37°C, 5% $CO_2$ culture box following the same process described previously, which was followed by the addition of 100 nM recombinant IL-2 to induce $CD4^+$ T cell differentiation.

*Co-culture of peripheral blood $CD4^+$ T cells with recombinant adenovirus PAD-JAZF1*

Following the procedure described previously herein, the cellular extract was collected and the $CD4^+$ T cell subtype (active, memory, restrictive T cells, and Tregs) and inflammatory factors IL-4, IL-10, IFN-γ (3 h), and IL-6 (6 h) were assessed by flow cytometry.

**Animals and *in vivo* adenovirus infection**

A high-fat diet (HFD) mouse model was employed to assess the role of *JAZF1 in vivo*. C57BL/6J mice (4 weeks of age; 10–18 g, n=14) were placed together in the same cage and fed a regular diet (RD) for 1 week before the experiment. The temperature and humidity were 18–25°C and 40–60%, respectively, with a 12-h light–dark cycle. Water and food were withheld from all mice during the experiment. All mice were divided into PAD-JAZF1 (treatment group, n=6) and pAdEasy (control group, n=6) groups after 1 week. Moreover, mice in both groups were fed a high-fat diet (HFD). Another 2 mice, as a blank control, were fed a RD. After feeding for 12 weeks, the vectors were introduced into the C57BL/6J mice separately via tail vein injection. Three days later, mice were killed by cervical dislocation, and adipose tissues were harvested from the testicles or uterus, kidney, and abdomen. Muscle and liver tissues were also acquired. The 3 kinds of tissue were used for Western blotting and RT-PCR to detect the expression of *JAZF1*. The liver tissues were also used for hematoxylin & eosin staining and immunohistochemistry (IHC). By detecting $CD11c^+$ macrophages, $CD4^+$ T cells, and TNF-α, IL-6, and IL-10, the effects of *JAZF1* on chronic inflammation in liver tissues in HFD mice were investigated.





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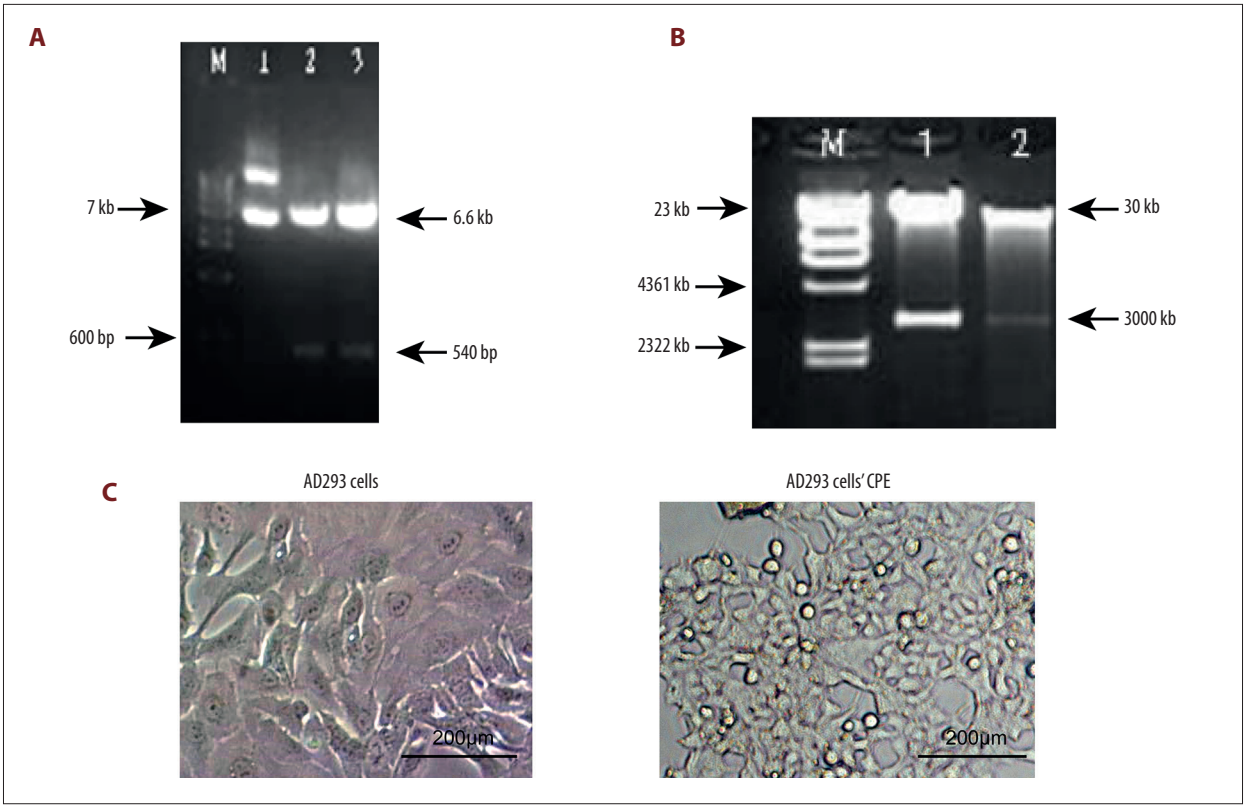


**Figure 1.** Restriction mapping and cytopathic effect (CPE) on AD293 cells transfected with the adenoviral plasmid. (**A**) Shuttle plasmid pShuttle-JAZF1 (lane M): wide-range DNA marker; (lane 1): Recombinant plasmid pShuttle (6.6 kb); (lane 2, 3): pShuttle-JAZF1 digested by XhoI and EcoRI (540 bp). (**B**) Recombinant plasmid pAD-JAZF1 (lane M): λhindIII digestion, up to 23 kb; (lane 1, 2): pAd-JAZF1 digested by PacI. (**C**) AD293 cells and the CPE.

### JAZF1 expression measured by Western blotting and RT-PCR

Total liver, muscle, and adipose tissue proteins were extracted and used for SDS-PAGE at 80 V for 30 min and 120 V for 90 min. After transfer to a membrane (wet), a primary antibody (rabbit anti-mouse *JAZF1* polyclonal antibody; Abcam, Cambridge, UK) was added and incubated at 4°C in a refrigerator overnight, which was followed by washing. The secondary antibody (HRP-sheep-rabbit-IgG; Abcam) was diluted (1: 1000) and incubated at room temperature for 70–80 min. After washing, Quantity One software (BIO-RAD, Hercules, CA, USA) was used for imaging and IOD values were calculated. By calculating the ratio of each value to the IOD value of internal controls, the relative expression of the target protein was determined.

For RT-PCR, mRNA extraction and reverse transcription were performed using the TAKARA mRNA Extraction and Reverse Transcription Kit. The reaction conditions were as follows: 37°C for 15 min, 85°C for 5 s, and 4°C for 20 min. PCR was performed immediately using a PCR instrument (Hangzhou Anjes) with the following primers for *JAZF1*: 5'-CACGCCGAGAACAGGAAT-3' and 5'-GTGCTGCTGCGGAATGAA-3'. β-*actin* was used as the internal reference. The specificity and relative amplification efficiency were evaluated using Bio-Rad CFX Manager 2.0.

### HE and IHC staining to detect the effect of JAZF1 on chronic inflammation

Fresh liver tissues were obtained from mice for HE and IHC (using reagents provided with a kit from China Biyuntian Biotechnology Co., Shanghai, China). The effects of *JAZF1* on chronic inflammation were investigated by assessing $CD11c^+$ macrophages, $CD4^+$ T cells, TNF-α, IL-6, and IL-10.

### Statistical analysis

Data are presented as means±SEM and the differences between groups were calculated using independent-samples *t* tests. $P<0.05$ indicated significance. SPSS software 19.0 (IBM, Armonk, NY, USA) was used to conduct all statistical analyses.





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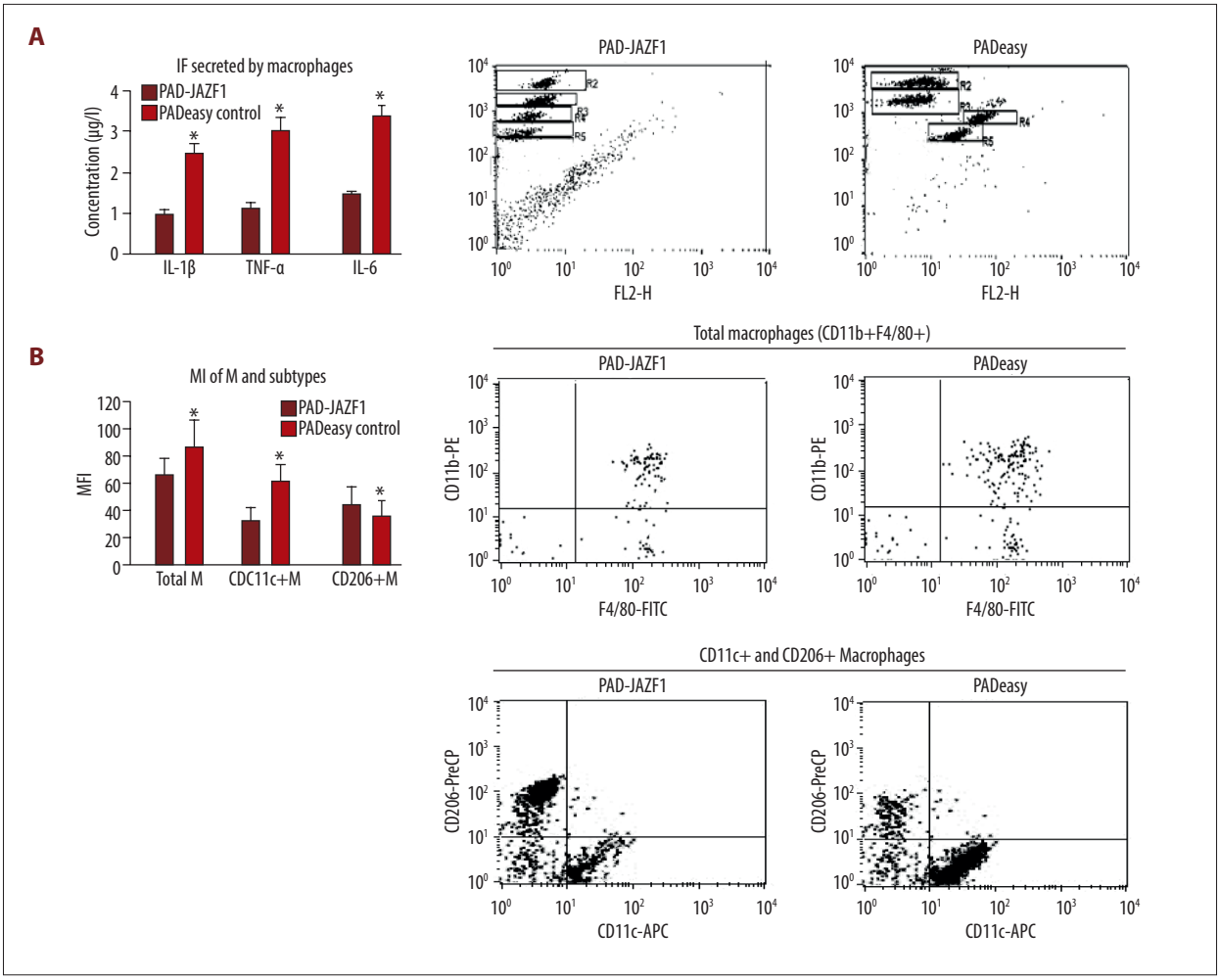


**Figure 2.** Cell populations and secretion levels in mouse peritoneal macrophages and subtypes in extracts obtained from co-culture conditions. (**A**) Inflammation-related factors secreted by mouse peritoneal macrophages. Flow cytometry results of IL-1β (gate R3), IL-6 (gate R4), and TNF-α (gate R5) in each group. (**B**) Mouse peritoneal macrophages and subtype populations. Values are presented as means±SEM (n=6/group). * P<0.05 *vs.* the pAdEasy control group.

## Results

### Construction of an adenovirus vector to overexpress JAZF1 (PAD-JAZF1)

The Pshuttle-JAZF1 was digested by XhoI and EcoRI double-enzyme digestion and analyzed on agarose gels to identify these fragments (Figure 1A). Two fragments of 6.6 kb and 540 bp were detected, corresponding to pShuttle and the insertion fragment, and the sequences were verified. Using PacI, PAD-JAZF1 was digested into 2 fragments of 30 kb (adenovirus skeleton plasmid) and 3.0 kb (PAD-JAZF1) (Figure 1B), and the inserted fragments were confirmed by sequencing.

CPE assays were performed to determine whether increased viral protein expression led to enhanced cell killing. The infected AD293 cells exhibited an irregular shape consistent with CPE (Figure 1C). A plaque formation assay showed that the virus titers were $7.0\times10^8$ and $8.0\times10^8$ pfu/mL for PAD-JAZF1 and pAdEasy, respectively.

### Recombinant adenovirus PAd-JAZF1 inhibits mouse peritoneal macrophage differentiation towards CD11c⁺ M and suppresses the secretion of inflammatory factors *in vitro*

Mouse peritoneal macrophages were cultured with the recombinant adenovirus PAD-JAZF1 and airborne adenovirus pAdEasy. After adding LPS (3 or 6 h), inflammatory factors IL-1β, TNF-α (3 h), and IL-6 (6 h), as well as macrophage subtypes (CD11c⁺ and CD206⁺), were detected by flow cytometry. The CD11c⁺M population and secretion of inflammatory factors were significantly lower and the CD206⁺ M population was larger in the PAD-JAZF1 group than in the control group. These results





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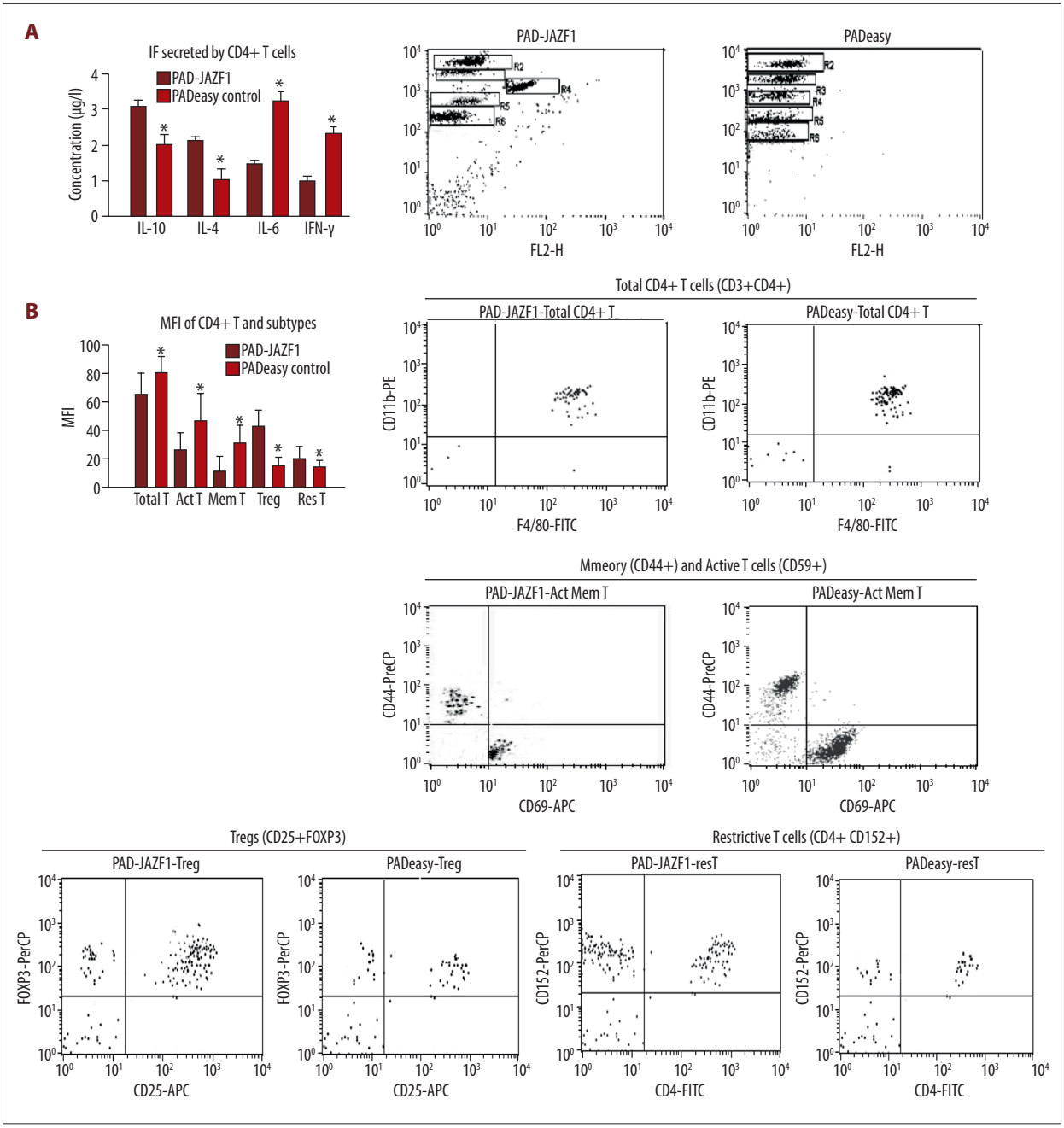


**Figure 3.** Cell populations and secretion of inflammatory factors by mouse CD4⁺ T cells and subtypes in co-culture conditions. (**A**) Inflammatory factors secreted by CD4⁺ T cells. Flow cytometry results for IL-6 (gate R3), IL-10 (gate R4), IFN-γ (gate R5), and IL-4 (gate R6) in each group. (**B**) Mouse CD4⁺ T cells and their subtypes. Values are presented as means±SEM (n=6/group). * P<0.05 *vs.* the pAdEasy control group.

indicated that *JAZF1* inhibited macrophage differentiation into the CD11c⁺M lineage, as well as the secretion of inflammatory factors, *in vitro* (Figure 2).

### Recombinant adenovirus PAd-JAZF1 regulates mouse CD4⁺ T cell differentiation and suppresses the secretion of inflammatory factors *in vitro*

Mouse CD4⁺ T cells were cultured with the recombinant adenovirus PAD-JAZF1 and airborne adenovirus pAdEasy. After adding recombinant IL-2 stimulus (3 or 6 h), CD4⁺ T cell subtypes





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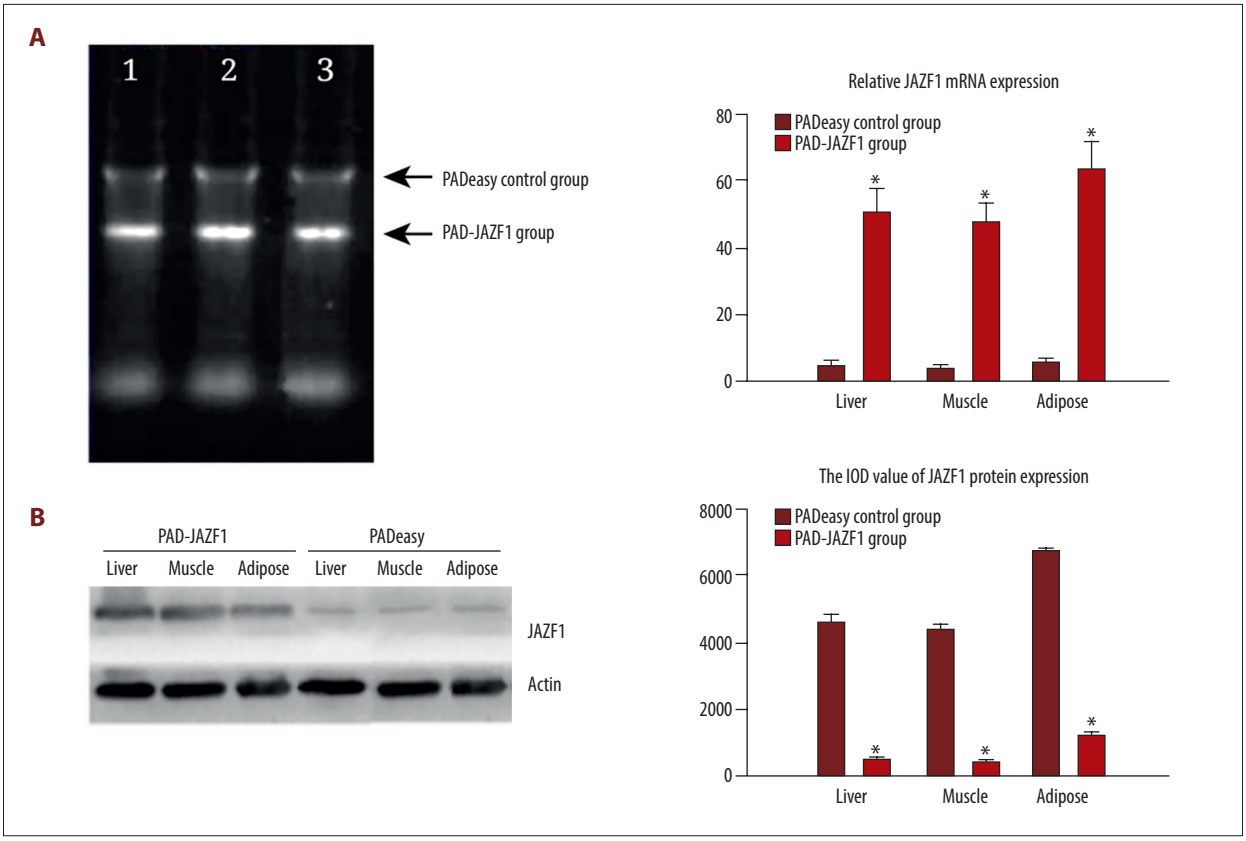


**Figure 4.** mRNA and protein expression levels of JAZF1 in mouse liver, muscle, and adipose tissues in the PAD-JAZF1 and control groups. (**A**) *JAZF1* mRNA expression levels in the 2 groups. Lane 1, liver; lane 2, muscle; lane 3, adipose tissue 4. (**B**) JAZF1 protein expression levels in the PAD-JAZF1 and control groups.

(active, memory, restrictive T cells, and Tregs) and inflammatory factors (IL-4, IL-10, and IFN-γ (3 h) and IL-6 (6 h)), were detected by flow cytometry. The populations of total $CD4^+$ T cells, active T cells, and memory T cells and the secretion of IL-6 and IFN-γ were suppressed in the PAD-JAZF1 group, but Tregs, restrictive T cells, IL-10, and IL-4 were elevated in the PAD-JAZF1 group (Figure 3).

### Effects of the recombinant adenovirus (PAD-JAZF1) on JAZF1 mRNA and protein expression in mouse adipose, liver, and muscle tissues

After mouse tail vein injection of recombinant adenovirus (pad-JAZF1, pAdEasy) for 3 days, mouse adipose, liver, and muscle tissues were collected to evaluate the expression of *JAZF1* at the mRNA and protein levels. By RT-PCR and Western blotting, *JAZF1* mRNA and protein levels in the PAD-JAZF1 group were significantly higher than those in the pAdEasy infection group (Figure 4), demonstrating that PAD-JAZF1 can increase *JAZF1* transcription and translation in each tissue type.

### JAZF1 decreases $CD11c^+$ macrophages and suppresses TNF-α and IL-1β secretion *in vivo*

*JAZF1* inhibited the number of and secretion by peritoneal macrophages *in vitro*. To determine whether *JAZF1* has similar effects *in vivo*, we transfected PAD-JAZF1 into HFD-fed mice. Liver IHC results showed that $CD11c^+$ macrophages and TNF-α and IL-1β levels in the PAD-JAZF1 group were lower than those in the control group, suggesting that *JAZF1* can downregulate $CD11c^+$M and suppress secretion *in vivo*. To demonstrate the chronic inflammation status in HFD-fed mice, we performed liver HE staining (using RD mice as a control group). Liver tissue inflammation was significantly greater in HFD mice than in RD mice (Figure 5).

### JAZF1 affects $CD4^+$ T cell populations and cytokine secretion *in vivo*

*JAZF1* inhibited the number of and secretion by $CD4^+$ T cells *in vitro*. We further evaluated the effects of *JAZF1 in vivo* by transfecting PAD-JAZF1 into HFD-fed mice. Liver IHC results showed that the $CD4^+$ T cell population and the secretion of IL-6 in the PAD-JAZF1 group were lower than those in the pAdEasy control





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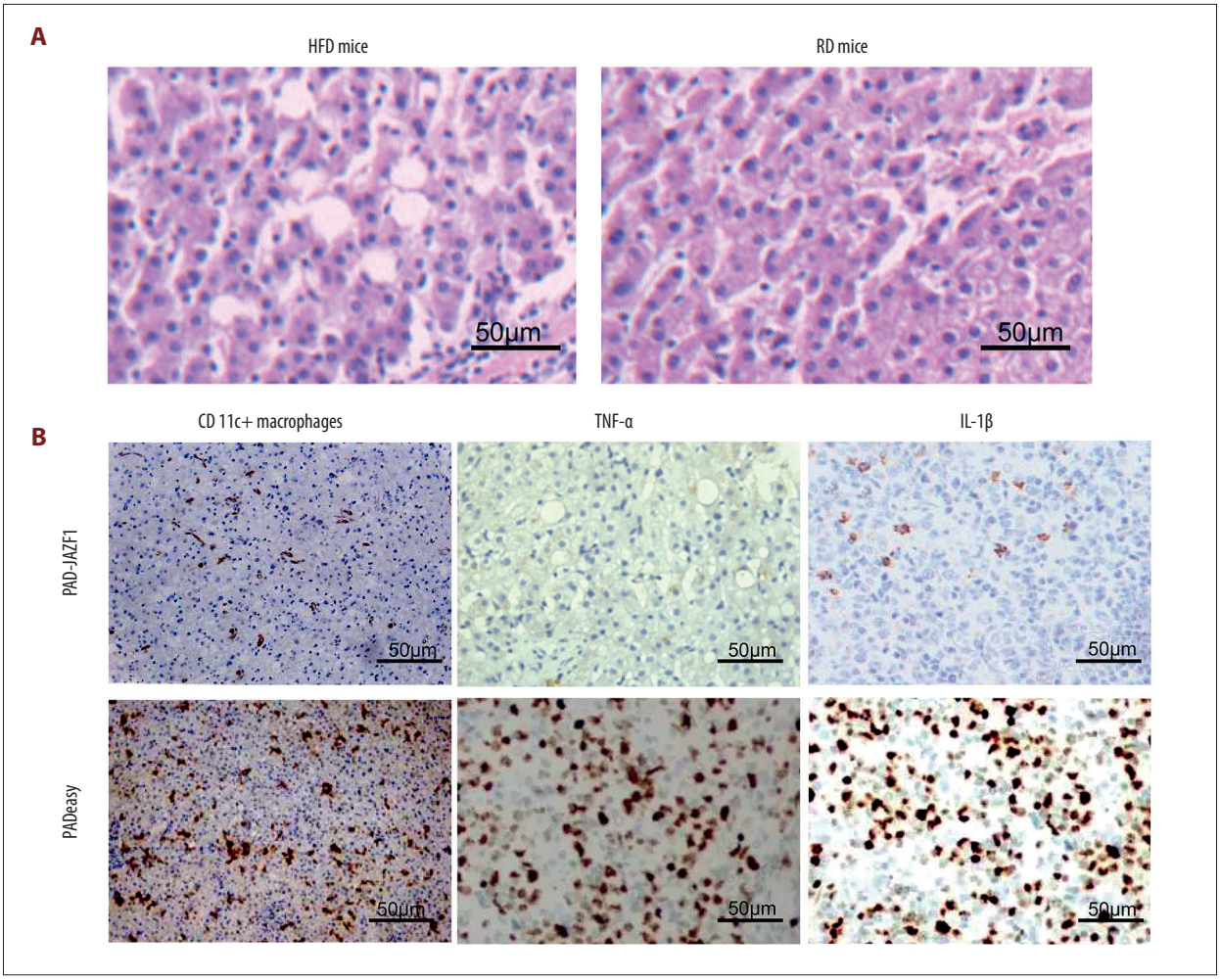


**Figure 5.** CD11c⁺ macrophages and TNF-α and IL-1β expression levels in the liver. (**A**) HE staining results for the liver of high-fat diet (HFD)-fed mice and regular diet (RD)-fed mice. (**B**) IHC staining results for CD11c⁺ macrophages and TNF-α and IL-1β levels in the livers of the PAD-JAZF1 group and pAdEasy control group.

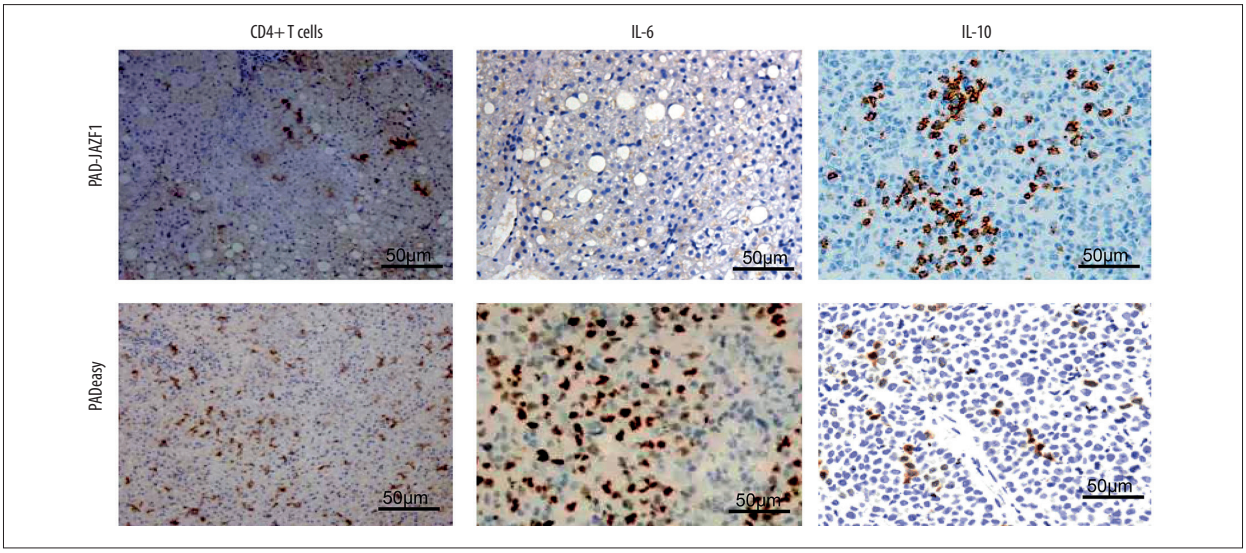


**Figure 6.** IHC analysis of CD4⁺ T cells and IL-6 and IL-10 expression levels in the livers of the PAD-JAZF1 and pAdEasy control groups.





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group, but that IL-10 levels were higher. These results indicate that *JAZF1* can regulate the liver CD4+ T cell populations and secretion *in vivo* (Figure 6).

## Discussion

Gluconeogenesis, lipid metabolism, insulin sensitivity, islet β cell function, and inflammatory reactions are regulated by *JAZF1* [8–11]. Glucose tolerance and insulin sensitivity are elevated in mice with *JAZF1* overexpression, suggesting that this gene affects the regulation of insulin resistance [12]. The overexpression of *JAZF1* can inhibit the release proinflammatory cytokines through the inhibition of stress-activated protein kinases and nuclear factor-κB, which is accompanied by a decrease in the activation of c-Jun N-terminal kinase, supporting the important role of this gene in preventing lipogenesis and systemic inflammation-related diseases [13]. However, the role of *JAZF1* in chronic inflammation is unclear. In this study, RT-PCR and Western blotting showed that *JAZF1* was successfully transfected into the C57BL/6J mice, with high expression in the liver, muscle, and adipose tissues. We found that *JAZF1* reduces chronic inflammation by limiting macrophage and CD4+ T cell populations, altering subtype differentiation, and regulating the secretion of inflammatory factors *in vitro*. Furthermore, we found that *JAZF1* relieved the chronic inflammation of hepatocytes *in vivo*.

Adipose tissue can secrete a variety of fat-specific inflammatory factors and is involved in glycolipid metabolism [2,4], which mediates macrophage activation, promotes inflammation, and leads to insulin resistance. Inflammatory factors such as TNF-α in the innate immune system released by macrophages phagocytosing necrotic adipose cells hinder the function of the remaining adipose cells. Free fatty acids released from dysfunctional adipose cells can then activate macrophages [14]. This suggests that the interaction between fat cells and macrophages can trigger a cycle of inflammatory reactions. The M1 lineage leads to inflammation and insulin resistance [15–18], but CD206+ macrophages are predominantly anti-inflammatory [19,20]. In tissues with local inflammation, M2 (expressing CD206+) macrophages tend to transform into the M1 type (expressing CD11c+) [21]. In this study, the populations of total macrophages and CD11c+ macrophages and the secretion of proinflammatory cytokines were reduced in the PAD-JAZF1 group. The results of our *in vitro* and *in vivo* analyses are consistent with those of our previous studies and demonstrate that *JAZF1* overexpression can decrease MHCII, CD40, and CD86 in total ATM, CD11c+ATM, and CD206+ATM cells, thereby restricting antigen presentation [6]. These findings suggest that *JAZF1* is essential for the role of macrophages in inflammatory responses. The mechanism underlying these effects will be explored in future studies.

Low-grade chronic inflammation contributes to systemic metabolic diseases such as T2D and is associated with immune disorders. The proinflammatory response is further associated with an imbalance in T cell subtypes, and particularly CD4+ T cell subtypes [22]. CD4+ T cells are important for obesity-associated diseases, and adipocytes, as antigen-presenting cells, regulate CD4+ T cell activity [22]. In immunodeficient Rag1$^{-/-}$ and H2A$^{-/-}$ mice, CD4+ T cells mediate obesity memory and promote weight regain, and the depletion of CD4+ T cells leads to obesity memory ablation [22]. In adipose tissues of obese individuals, the Th1-type immune environment dominates the Th2/Treg-type due to the overproduction of proinflammatory cytokines (IFN-γ, IL-6, and TNF-α) and a deficiency in Th2-type processes and interleukins (IL-4, IL-5, IL-10, and IL-13). ILCs2 secrete mostly IL-4, IL-5, IL-9, and IL-13, which are responsible for the accumulation of eosinophils and the polarization of alternatively-activated macrophages, creating a beneficial anti-inflammatory and metabolic regulatory environment in the adipose tissue [23]. These findings indicate that the differentiation of CD4+ T cell subtypes is closely related to lipid metabolism. The expression of genes related to lipid metabolism and the inhibition of lipid accumulation in adipocytes can be regulated by *JAZF1* [24]. In this study, the effects of *JAZF1* were evaluated *in vitro* using CD4+ T cells and *in vivo* using HFD-fed mice. The recombinant adenovirus PAd-JAZF1 regulated mouse CD4+ T cell differentiation and secretion *in vitro* and *in vivo*.

Tregs, a subtype of CD4+ T cells, have inhibitory effects on immune and inflammatory reactions, especially on the response to an individual's own antigens [25,26]. They act as anti-inflammatory cells to counter the proinflammatory immune cells responsible for obesity-induced inflammation [27]. A decrease in Tregs will cause metabolic disorders in adipose tissue and insulin resistance [28]. The imbalance of regulatory T (Treg) cells contributes to tissue-specific and systemic inflammation and immunity in T2D, and restoration of the Treg balance is a potential therapeutic approach for prevention and treatment [29]. This is consistent with observations in patients with T2D who are obese and have insulin resistance [30]. Based on these previous findings, the interaction between *JAZF1* and Tregs and the effect of *JAZF1* overexpression on chronic inflammation warrants further study.

## Conclusions

In conclusion, co-culture with PAD-JAZF1 affected cell populations and secretion status. *In vivo*, mice transfected with PAD-JAZF1 showed lower levels of CD11c+ M and CD4+ T cells and reduced proinflammatory cytokine secretion. Our results show that *JAZF1* can reduce chronic inflammation by limiting macrophage and CD4+ T cell populations, adjusting subtype differentiation, and regulating the secretion of inflammatory factors.





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## Conflict of interest

None.

## References:

1. Chatterjee S, Khunti K, Davies MJ: Type 2 diabetes. Lancet, 2017; 389: 2239–51
2. Jafarirad S, Ayoobi N, Karandish M et al: Dark chocolate effect on serum adiponectin, biochemical and inflammatory parameters in diabetic patients: A randomized clinical trial. Int J Prev Med, 2018; 9: 86
3. Redwine LS, Pung MA, Wilson K et al: Differential peripheral inflammatory factors associated with cognitive function in patients with heart failure. Neuroimmunomodulation, 2018; 25: 146–52
4. Li C, Xu MM, Wang K et al: Macrophage polarization and meta-inflammation. Transl Res, 2018; 191: 29–44
5. Becker M, Levings MK, Daniel C. Adipose-tissue regulatory T cells: Critical players in adipose-immune crosstalk. Eur J Immunol, 2017; 47: 1867–74
6. Meng F, Lin Y, Yang M et al: JAZF1 inhibits adipose tissue macrophages and adipose tissue inflammation in diet-induced diabetic mice. Biomed Res Int, 2018; 2018: 4507659
7. Gong X, Zhang C, Yiliyasi-Aisa Y-A et al: A comparative analysis of genetic diversity of candidate genes associated with type 2 diabetes in worldwide populations. Yi Chuan, 2016; 38: 543–59
8. Wei Q, Zhou B, Yang G et al: JAZF1 ameliorates age and diet-associated hepatic steatosis through SREBP-1c -dependent mechanism. Cell Death Dis, 2018; 9: 859
9. Rasheed MA, Kantoush N, Abd El-Ghaffar N et al: Expression of JAZF1, ABCC8, KCNJ11and Notch2 genes and vitamin D receptor polymorphisms in type 2 diabetes, and their association with microvascular complications. Ther Adv Endocrinol Metab, 2017; 8: 97–108
10. Liao ZZ, Wang YD, Qi XY, Xiao XH: JAZF1, a relevant metabolic regulator in type 2 diabetes. Diabetes Metab Res Rev, 2019; 35: e3148
11. Hebbar P, Abubaker JA, Abu-Farha M et al: A perception on genome-wide genetic analysis of metabolic traits in Arab populations. Front Endocrinol (Lausanne), 2019; 10: 8
12. Yuan L, Luo X, Zeng M et al: Transcription factor TIP27 regulates glucose homeostasis and insulin sensitivity in a PI3-kinase/Akt-dependent manner in mice. Int J Obes (Lond), 2015; 39: 949–58
13. Yang M, Dai J, Jia Y et al: Overexpression of juxtaposed with another zinc finger gene 1 reduces proinflammatory cytokine release via inhibition of stress-activated protein kinases and nuclear factor-kappaB. FEBS J, 2014; 281: 3193–205
14. Nguyen KD, Qiu Y, Cui X et al: Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. Nature, 2011; 480: 104–8
15. Lumeng CN: Adipose tissue macrophages: A piece of the PAI of metabolic syndrome. Sci Transl Med, 2010; 2: 20ps7
16. Lumeng CN, Bodzin JL, Saltiel AR: Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest, 2007; 117: 175–84
17. Lumeng CN, Deyoung SM, Bodzin JL, Saltiel AR: Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. Diabetes, 2007; 56: 16–23
18. Lumeng CN, Saltiel AR: Inflammatory links between obesity and metabolic disease. J Clin Invest, 2011; 121: 2111–17
19. Fujisaka S, Usui I, Ikutani M et al: Adipose tissue hypoxia induces inflammatory M1 polarity of macrophages in an HIF-1alpha-dependent and HIF-1alpha-independent manner in obese mice. Diabetologia, 2013; 56: 1403–12
20. Kambara K, Ohashi W, Tomita K et al: *In vivo* depletion of CD206+ M2 macrophages exaggerates lung injury in endotoxemic mice. Am J Pathol, 2015; 185: 162–71
21. Pejnovic NN, Pantic JM, Jovanovic IP et al: Galectin-3 deficiency accelerates high-fat diet-induced obesity and amplifies inflammation in adipose tissue and pancreatic islets. Diabetes, 2013; 62: 1932–44
22. Jagannathan-Bogdan M, McDonnell ME, Shin H et al: Elevated proinflammatory cytokine production by a skewed T cell compartment requires monocytes and promotes inflammation in type 2 diabetes. J Immunol, 2011; 186: 1162–72
23. Chalubinski M, Luczak E, Wojdan K et al: Innate lymphoid cells type 2 – emerging immune regulators of obesity and atherosclerosis. Immunol Lett, 2016; 179: 43–46
24. Ming GF, Xiao D, Gong WJ et al: JAZF1 can regulate the expression of lipid metabolic genes and inhibit lipid accumulation in adipocytes. Biochem Biophys Res Commun, 2014; 445: 673–80
25. Sun L, Fu J, Zhou Y: Metabolism controls the balance of Th17/T-regulatory cells. Front Immunol, 2017; 8: 1632
26. Han JM, Patterson SJ, Speck M et al: Insulin inhibits IL-10-mediated regulatory T cell function: Implications for obesity. J Immunol, 2014; 192: 623–29
27. Lee BC, Lee J: Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. Biochim Biophys Acta, 2014; 1842: 446–62
28. Feuerer M, Herrero L, Cipolletta D et al: Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med, 2009; 15: 930–39
29. Wang M, Chen F, Wang J et al: Th17 and Treg lymphocytes in obesity and Type 2 diabetic patients. Clin Immunol, 2018; 197: 77–85
30. Reilly SM, Saltiel AR: Adapting to obesity with adipose tissue inflammation. Nat Rev Endocrinol, 2017; 13: 633–43





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