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Adiponectin deficiency has no effect in murine autoimmune myocarditis

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ABSTRACT

Background: Adiponectin is a hormone that together with its receptors modulates a number of metabolic processes including gluconeogenesis and lipid catabolism. It belongs to the C1QTNF (complement C1q tumor necrosis factor-related protein) family, which has a variety of members with high amino acid sequence homology and overlapping functions. Concentration of adiponectin in blood is inversely correlated with body fat percentage and cardiac risk factors like blood pressure and CRP (C-reactive protein) level. Studies have identified the existence of a cardiac adiponectin system. However, little is known about the role of this system in the pathogenesis of autoimmune myocarditis. Thus, we have studied the involvement of adiponectin in the development of this autoimmune disorder in a mouse model of experimental autoimmune myocarditis (EAM).

Methods: Adiponectin knockout (ko) and wild type (wt) mice were immunized with cardiac troponin I (cTnI) to induce an EAM. To determine the severity of myocardial damage, inflammation and fibrosis were scored after HE and Afog staining and high sensitivity troponin T (hsTnT) level was measured. To detect if changes in specific inflammatory cell numbers could be observed between the genotypes, we performed immunohistochemical staining to detect T lymphocytes, B lymphocytes and macrophages. The level of the humoral immune response was determined through the measurement of cTnI-specific serum IgG autoantibodies. Relative mRNA expression of different cytokines, C1QTNF family members and adiponectin receptors in the heart tissue was analyzed with qPCR.

Results: Animals immunized with cTnI developed autoimmune myocarditis with a significant deterioration of cardiac parameters compared to the corresponding control group. The adiponectin ko group immunized with cTnI showed a tendency towards increased inflammation, fibrosis, heart-to-body-weight ratio, infiltration pattern of T lymphocytes, B lymphocytes and macrophages, hsTnT concentration, humoral immune response and mRNA expression of interleukin 6 in the heart tissue and decreased weight gain compared to the wt group immunized with cTnI. However, the difference to the wt group treated with cTnI was not significant. The analysis of cardiac mRNA expression of adiponectin receptors and four C1QTNF family members, most suitable for fulfilling the functions of adiponectin in adiponectin ko mice, did not show any significant differences between adiponectin ko and wt group at all.

Conclusion: Our study reveals that the absence of adiponectin did not lead to a significantly increased impairment of cardiac function and was also unlikely to be compensated by its receptors or other C1QTNF family members in the murine model of EAM. Here, other synergistic or redundant effects might play a role and must be investigated in further studies to understand the role and function of adiponectin in autoimmune myocarditis.

1. Introduction

Myocarditis is an inflammation of the myocardium, which can be caused by virus, bacteria, medications, radiation or autoimmune reactions [1]. The course of disease can be mild and asymptomatic but also fulminant and fatal. In autopsies of young adults after sudden deaths, myocarditis is found in up to 9 % of cases [2]. Its therapy is often symptomatic and involves prophylaxis of thromboembolism and treatment of complications. A causal therapy, such as antiviral or immunosuppressive therapy, is usually carried out only within the

Abbreviations: cTnI, cardiac troponin I; C1QTNF, complement C1q tumor necrosis factor-related protein; DCM, dilated cardiomyopathy; EAM, experimental autoimmune myocarditis; hsTnT, high sensitivity troponin T

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framework of studies, since the only reliable method for the diagnosis is a myocardial biopsy. Chronic courses account for 10–20 % of the cases and can lead to dilated cardiomyopathy (DCM) accompanied by a heart failure [2]. DCM is characterized by a ventricular enlargement followed by cardiomegaly and reduction in systolic ejection fraction. According to statistics only half of the affected patients survive five years following diagnosis [3].

Studies of DCM have shown the existence of a cardiac adiponectin system, which seems to be involved in pathogenesis of this disease. Adiponectin plays a modulating role in the inflammatory process of virus induced myocarditis [4]. Adiponectin is an adipocytokine. It has numerous functions in the human body which are exerted via adiponectin receptor 1 and 2 [5]. Adiponectin is involved in cell metabolism, apoptosis control and immunomodulation and has an antiinflammatory, antithrombotic, antiatherogenic and cardioprotective effect both *in vitro* and in animal experiments [6–12]. Furthermore, it belongs to the complement C1q tumor necrosis factor-related protein (C1QTNF) family, which has a variety of members with high amino acid sequence homology and complementary functions [13]. C1QTNF9 is the closest molecule to adiponectin. The isoforms share 98 % amino acid identity. Like adiponectin, C1QTNF9 is able to exert vasodilatory and vasoprotective effects via AMP-activated protein kinase (AMPK)/Protein kinase B/endothelial nitric oxide synthase (eNOS) pathway [13,14]. Other members of the family such as C1QTNF3 and C1QTNF6 have antiinflammatory effects. For example, C1QTNF3 inhibits lipopolysaccharide (LPS) and toll-like receptor (TLR)-mediated inflammation and C1QTNF6 enhances the expression of interleukin 10 (IL10) in monocytes and activates p42/44 mitogen-activated protein kinase cascade. C1QTNF1, which expression is supposed to be upregulated by inflammatory cytokines and in adiponectin knockout (ko) mice, is highly expressed in the heart [15–17].

Like the other members of the C1QTNF family, adiponectin exerts its antiinflammatory effect in many ways. It stimulates the production of antiinflammatory cytokines, e.g. IL10 and IL1 receptor antagonist in monocytes, macrophages and dendritic cells [6,8,9]. In addition, it reduces the synthesis of C-reactive protein (CRP), IL17 and interferon gamma (IFNγ). Moreover, adiponectin inhibits the TLR 4 signaling pathway, IL2-induced natural killer cell (NK cell) activation and tumor necrosis factor (TNF)-related apoptosis inducing ligand [6,7,11,18–20].

Apart from its function in other autoimmune diseases [21], still little is known about the role of adiponectin in the pathogenesis of autoimmune myocarditis. Thus, we studied its role in the pathogenesis of autoimmune cardiomyopathies using our model of cTnI-induced experimental autoimmune myocarditis (EAM).

2. Methods

2.1. Experimental setting

Adiponectin ko mice were kindly provided by PD Dr. med. C. Skurk and backcrossed to A/J background for at least six generations. Both adiponectin ko and wild type (wt) mice were maintained in the animal facility unit of the University of Heidelberg, Germany. Five to six weeks old female mice were used in all experiments. The Animal Care and Use Committee of the University of Heidelberg approved all procedures involving the use and care of animals. Experiments were conducted according to the guidelines formulated by the European Community for experimental animal use (Directive 2010/63/EU).

Before the beginning of the experiment, the genotype of all animals was determined by PCR. DNA was isolated from tail biopsies followed by PCR using Type-it Mutation Detect PCR Kit (Qiagen, Hilden, Germany). The kit was used according to manufacturer's instruction. However, the number of cycles was adjusted to 40 and the annealing step was performed at 66 °C for 90 s. Primer sequences are listed in Table 1.

In the first part of the experiment, mice were treated with a subcutaneous injection of 100 µL emulsion, which contained either 150 µg cTnI-peptide (cTnI) in supplemented complete Freund's adjuvant with 5 mg/mL of *Mycobacterium tuberculosis* H37Ra (Sigma, St Louis, MO, USA) or adjuvant and control-buffer (phosphate-buffered saline (PBS)) alone. Mice treated with adjuvant and control-buffer alone served as control group. Two further immunizations were carried out 7 and 14 days after the first immunization. Mice were sacrificed on day 28 by cervical dislocation and subsequent heart explantation for further diagnosis.

Before sacrifice, blood samples were collected from each mouse retroorbitally under general anesthesia Ketamine:Xylazine (120 mg/kg:16 mg/kg intra peritoneal).

Table 1
Primer sequences of executed experiments.

Gene	Forward primer 5′- > 3′	Reverse primer 3′- > 5′
Primer sequences used for genotyping		
Adiponectin_ko	tggatgctgccatgttcccat	ctccagactgccttggga
Adiponectin_wt	tggatgctgccatgttcccat	cttgtgtctgtgtctaggcctt
Primer sequences used for qPCR		
Adiponectin	ggagagaaaggagatgcaggt	ctttcctgccaggggttc
Adiponectin receptor 1	gtttgccactcccaagca	acaccactcaagccaagtcc
Adiponectin receptor 2	tctcagtgggacatgtttgc	aggcctaagcccacgaac
C1QTNF1	caattcctccaccccagat	cccctcggtcacctttct
C1QTNF3	caacggcaacacagtcttca	gccaacttcagcactgcat
C1QTNF6	gggttcctctgggcagtatt	gccacagattctccaaaggt
C1QTNF9	ggaagagatggacgagatgg	tcctggaccaccaggatg
IL6	gctaccaaactggatataatcagga	ccaggtagctatggtactccagaa
IL10	caaaggaccagctggacaac	tcatttccgataaggcttgg
IL17	tccagaaggccctcagacta	tgagcttcccagatcacaga
IFNγ	cctttggaccctctgacttg	agcgttcattgtctcagagcta
L32	gctgccatctgttttacgg	tgactggtgcctgatgaact
TNFα	ctgtagcccacgtcgtagc	ttgagatccatgccgttg

C1QTNF1 - complement C1q tumor necrosis factor-related protein 1, C1QTNF3 - complement C1q tumor necrosis factor-related protein 3, C1QTNF6 - complement C1q tumor necrosis factor-related protein 6, C1QTNF9 - complement C1q tumor necrosis factor-related protein 9, IL6 – interleukin 6, IL10 – interleukin 10, IL17 – interleukin 17, IFNγ - interferon gamma, L32 - 60S ribosomal protein L32, TNFα - tumor necrosis factor alpha.

2.2. Histopathology and immunohistochemistry

For histopathological evaluation hearts were fixed in formalin (10 %) and subsequently embedded in paraffin. Formalin fixed heart sections (3–5 µm) were cut and stained with hematoxylin and eosin (HE) to determine the level of inflammation or with Acid fuchsin orange G-stain (Afog) to assess the grade of fibrosis, using standard staining protocols and reagents.

HE and Afog stained sections were analyzed under light microscopy as follows: grade 0 - no inflammation/fibrosis; grade 1 - cardiac infiltration/fibrosis in up to 5 % of the cardiac sections; grade 2: 6–10 %; grade 3: 11–30 %; grade 4: 31–50 %; and grade 5: > 50 %. Histopathological evaluation was conducted by two independent examiners who were blinded to the treatment status of the respective groups.

For immunohistochemical detection of infiltrated T lymphocytes (CD4, CD8), B lymphocytes (CD45) and macrophages (CD68), serial sections of 0.5 µm thickness were cut. After heat-induced epitope retrieval for 30 min, sections were incubated with primary antibody (Table S1) overnight at 4 °C (CD8, CD45, CD68). Subsequently, the sections were washed and incubated with secondary antibody (horseradish peroxidase-coupled anti-rat IgG, 1:500) for 25 min followed by incubation with streptavidin horseradish peroxidase complex for 25 min. Antigens were visualized using Dako REAL Detection System. The detection of CD4 positive cells was performed using the Super-Vision 2 Red-single species rabbit AP kit according to the manufacturer's instructions. The incubation with primary antibody was

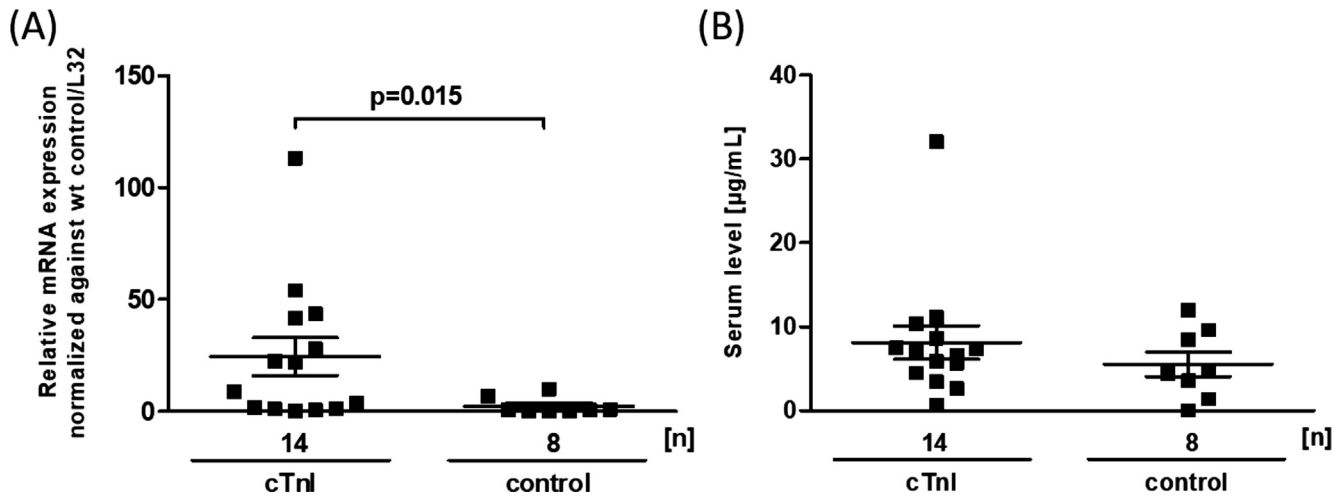

Fig. 1. Altered expression of adiponectin in EAM model. Female A/J mice were immunized with cTnI (n = 14) or control buffer (n = 8) and sacrificed on day 28. Relative mRNA expression of adiponectin was determined via qPCR (A). To detected adiponectin serum level, ELISA was performed (B). Data are depicted as means ± SEM. (Relative mRNA expression: wt/control: 2.44 ± 1.35 vs. wt/cTnI: 24.49 ± 8.40, p = 0.015; adiponectin serum level: wt/control: 5.52 ± 1.47 µg/mL vs. wt/cTnI: 8.11 ± 2.00 µg/mL).

carried out for 1 h at room temperature. For quantifying infiltrated leukocytes, four high power fields (40× magnification) were counted per slide and the number of positive cells was calculated per mm^2.

2.3. Body mass progression and heart-to-body-weight ratio

All animals were weighed on days 0 and 28. The body mass progression was determined as a change in body weight in percent. The hearts were removed and weighed in all animals on day 28 after the initial immunization. Heart-to-body-weight ratio was determined by the quotient of the heart weight and the body weight on the final day.

2.4. Determination of high sensitive troponin T (hsTnT) levels

Collected serum samples were diluted (1:10) with 0.9 % NaCl solution. The hsTnT level was measured by electrochemiluminescence method (ECLIA; Elecsys 2010 analyzer, Roche Diagnostics, Germany) [22].

2.5. Determination of cTnI autoantibody titers (immunoglobulin G, IgG)

Serum blood samples were collected from each mouse retroorbitally under general anesthesia. Autoantibody titers were measured by using ELISA technique. To measure serum cTnI antibody titers, plates were coated with 100 µL/well of cTnI (5 µg/mL) in bicarbonate buffer (pH 9.6) and incubated overnight at 4 °C. Blocking was performed with 1 % BSA/PBS. Serum samples were diluted to 1:800 followed by 1:4 dilution steps. Serum of untreated mice was used as control. Anti-mouse secondary antibody diluted to 1:5,000 for IgG (Sigma) was used for detection. Optical densities were determined at 450 nm. Antibody endpoint titers for each individual mouse were calculated as the greatest positive dilution of antibody yielding a positive signal and presented as 1/n.

2.6. Determination of adiponectin serum level

Collected serum samples were diluted (1:10,000) with 1 % BSA/PBS. The adiponectin level was measured using the Mouse Adiponectin/Acrp30 DuoSet ELISA (R&D systems, Minneapolis, USA) according to the manufacturer's protocol. Optical densities were determined at 450 nm.

2.7. Quantitative polymerase chain reaction (qPCR)

Quantitative PCR was performed to measure myocardial expression of various inflammation associated genes. Total RNA was extracted from heart tissue by using RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden Germany) according to the manufacturer's protocol, followed by cDNA synthesis using iScript™ gDNA Clear cDNA Synthesis Kit (Biorad, München, Germany). qPCR was carried out using iTaq™ SYBR Green Supermix (Biorad, München, Germany) according to the manufacturer's instructions with 75 ng cDNA. After an initial denaturation of 95 °C for 5 min, 40 cycles were performed consisting of denaturation at 95 °C for 10 s and annealing at 60 °C for 30 s.

Primer sequences used for quantification of transcript levels are listed in Table 1. Quantitative gene expression levels of all analyzed genes were normalized to the expression level of the housekeeping gene L32.

2.8. Statistical analysis

Results are expressed as mean ± SEM. Data were analyzed using two-way ANOVA (analysis of variance) followed by Bonferroni correction as post hoc analysis for the comparison of more than two groups. For two non-parametric groups, Mann-Whitney test was used. Values of $p < 0.05$ were considered statistically significant. Analysis was performed with GraphPad Prism 5.

3. Results

3.1. cTnI-immunization significantly increased cardiac mRNA expression level of adiponectin

To initially assess the expression of adiponectin during EAM induction (day 0 and 28), we determined the relative cardiac mRNA expression of adiponectin by qPCR as well as the adiponectin serum level by ELISA. Here, we could observe an increase of adiponectin mRNA expression in cTnI-immunized mice during time course of EAM, whereas control immunized mice showed a steady state adiponectin mRNA expression in the same period of time. In contrast to that, a decrease of serum level from day 0 to day 28 could be detected for cTnI-immunized as well as control treated mice (Fig. S1).

A detailed consideration of cardiac adiponectin mRNA at day 28 showed that cTnI-immunized mice had a significantly increased mRNA expression compared to control treated mice (Fig. 1A). In contrast to

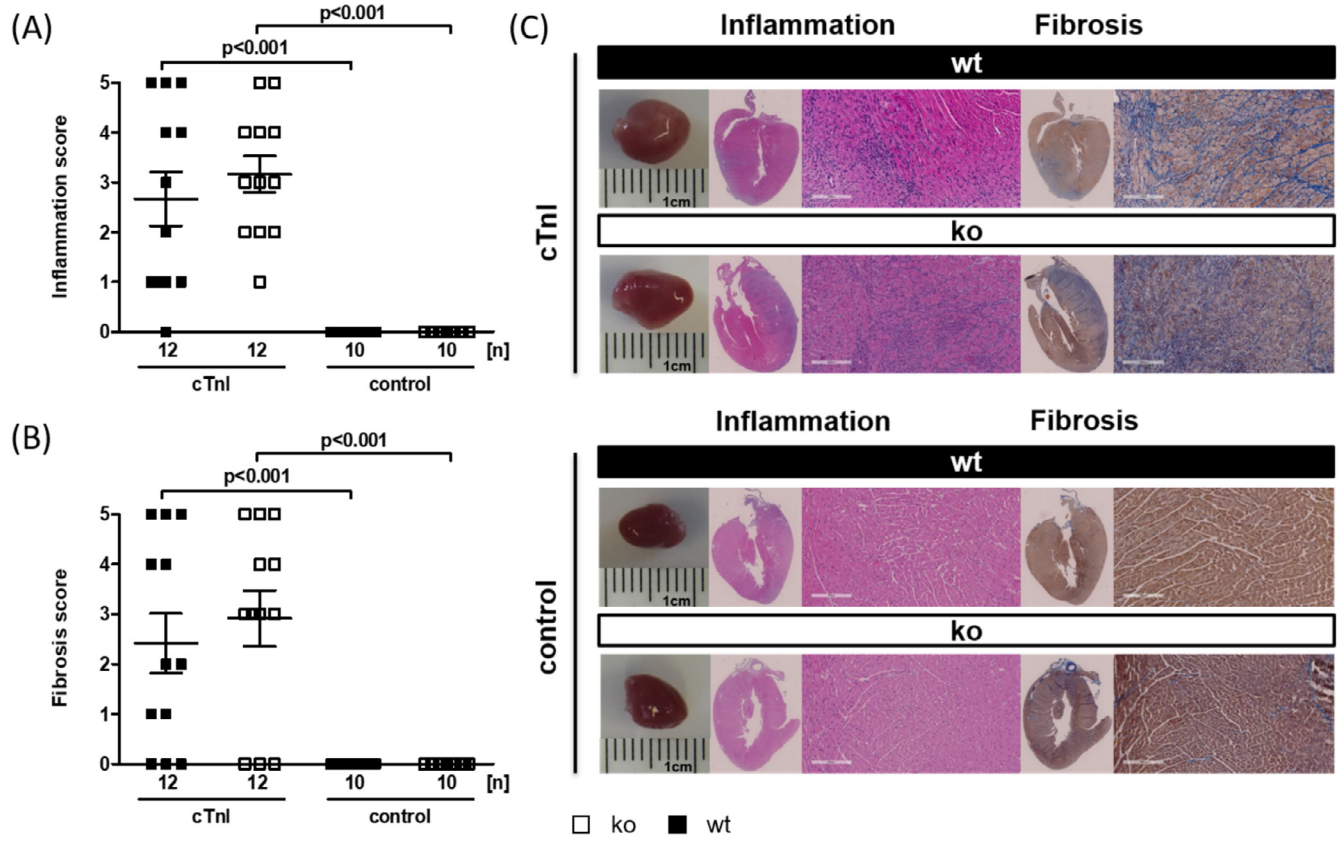

Fig. 2. Inflammation and fibrosis were not altered in adiponectin ko compared to corresponding wt mice. Adiponectin ko and corresponding wt mice were immunized with cTnI (n = 12) or control buffer (n = 10) and sacrificed on day 28. Inflammation and fibrosis score of hearts of immunized mice (A, B). Representative macroscopic pictures (column 1) and histopathological examinations (columns 2–5) of hearts stained with HE and Afog. Scale bars indicate 2 mm (column 2 and 4) and 200 µm (column 3 and 5) (C). (Inflammation score: wt/cTnI: 2.67 ± 0.54 vs. ko/cTnI: 3.17 ± 0.37, wt/control: 0 vs. ko/control: 0; fibrosis score: wt/cTnI: 2.42 ± 0.60 vs. ko/cTnI: 2.92 ± 0.56, wt/control: 0 vs. ko/control: 0).

that, no immunization-dependent variation of the adiponectin serum levels could be measured at day 28 (Fig. 1B).

3.2. Histological examination of hearts revealed no difference between adiponectin ko and wt mice

At day 28 after initiation of the experiment, hearts of all animals were removed for histological examination regarding inflammation (inflammation score) and fibrosis (fibrosis score). Control animals showed no signs of inflammation or fibrosis with no difference between wt and ko. cTnI-immunized mice developed significant myocardial inflammations and fibrosis compared to control groups ($p < 0.001$). However, no significant difference was detected between wt and ko animals (Fig. 2A and B). Fig. 2C shows representative cross-sections of hearts from cTnI-immunized and control mice stained with HE and Afog, respectively.

3.3. Infiltration pattern of T lymphocytes, B lymphocytes and macrophages was not altered between adiponectin ko and wt mice

To investigate the genotype dependent cardiac infiltration pattern of immune cells in the course of EAM, CD4 and CD8 T lymphocytes, B lymphocytes as well as macrophages were immunohistochemically differentiated. Here, the results showed a genotype independent infiltration pattern of CD4 and CD8 T lymphocytes, B lymphocytes and macrophages. However, big differences could be observed between the various cell types. Whereas the infiltration of B lymphocytes (CD45) and macrophages (CD68) was moderately increased, CD8 T lymphocytes showed hardly any infiltration. In contrast to that, CD4 T lymphocytes showed an obviously increased infiltration (Fig. 3).

3.4. Body mass progression and heart-to-body-weight ratio showed no difference between adiponectin ko and wt mice

To study the alterations of body mass in mice during the experiment, all animals were weighed on days 0 and 28. An increase in body mass was observed in almost all mice. The wt groups showed a stronger weight gain compared to adiponectin ko groups both after cTnI and control-buffer treatment. The difference was not significant (Fig. 4A).

Concerning the heart-to-body-weight ratio, the two groups treated with control-buffer showed no significant differences between each other. However, adiponectin ko mice treated with cTnI showed a significantly higher heart-to-body-weight ratio compared to the control group ($p < 0.05$). The adiponectin ko group immunized with cTnI showed a higher heart-to-body-weight ratio in comparison to the adiponectin wt group, but the difference between the two groups was not significant (Fig. 4B).

3.5. TnI autoantibody titers and hsTnT serum concentration was not altered between adiponectin ko and wt mice

In order to investigate the damage of cardiomyocytes, the concentration of hsTnT in sera was determined. The control group showed no hsTnT concentrations (detection limit < 30 pg/mL). The adiponectin ko group treated with cTnI showed a higher serum concentration of hsTnT compared to the adiponectin wt group, but the difference was not significant (Fig. 5A).

To study the effect of immunization on the humoral immune

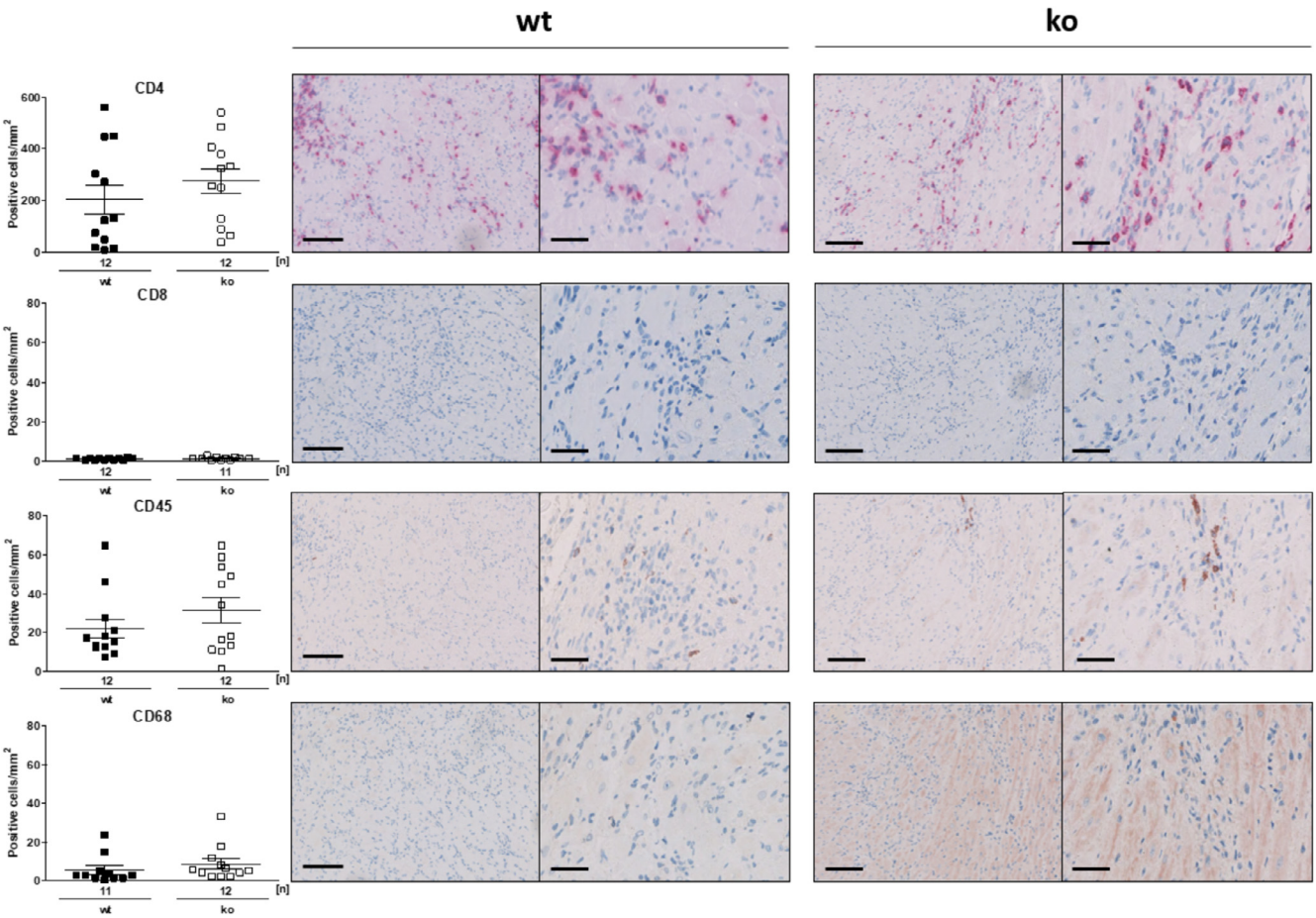

Fig. 3. Immunhistological stained heart sections of cTnI-immunized adiponectin ko and corresponding wt mice. Heart sections of adiponectin ko (n = 11–12) and corresponding wt mice (n = 11–12) were stained for infiltrated T lymphocytes (CD4, CD8), B lymphocytes (CD45) and macrophages (CD68). The number of positive cells per mm^2 was calculated (column 1). Scale bars indicate 100 μm (columns 2 and 4) and 50 μm (columns 3 and 5). (CD4: wt/cTnI: 205.40 ± 56.29 cells/mm^2 vs. ko/cTnI: 275.70 ± 47.88 cells/mm^2; CD8: wt/cTnI: 1.24 ± 0.14 cells/mm^2 vs. ko/cTnI: 1.56 ± 0.21 cells/mm^2; CD45: wt/cTnI: 21.95 ± 4.91 cells/mm^2 vs. ko/cTnI: 31.31 ± 6.35 cells/mm^2; CD68: wt/cTnI: 5.62 ± 2.17 cells/mm^2 vs. ko/cTnI: 8.74 ± 2.60 cells/mm^2).

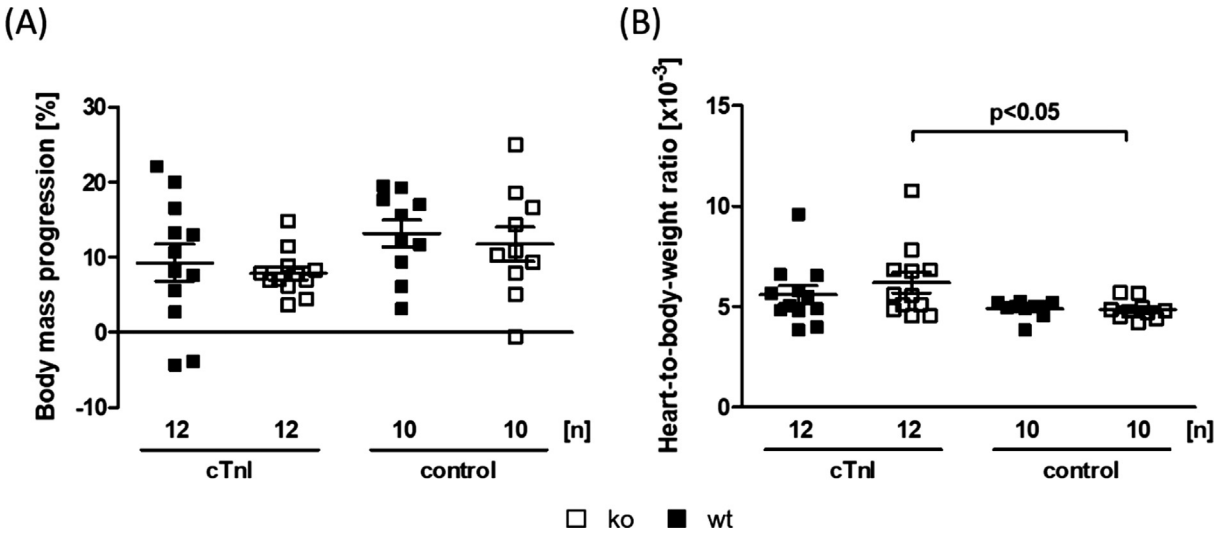

Fig. 4. Body mass progression and heart-to-body-weight ratio showed no difference in adiponectin ko compared to corresponding wt mice. Adiponectin ko mice and corresponding wt mice were immunized with cTnI (n = 12) or control buffer (n = 10) and sacrificed on day 28. Determination of body mass progression (A) and heart-to-body-weight ratio (B). (Body mass progression: wt/cTnI: 9.30 ± 2.43 % vs. wt/control: 13.18 ± 1.78 %, ko/cTnI: 7.86 ± 0.86 % vs. ko/control: 11.73 ± 2.29 %; heart-to-body-weight ratio: wt/cTnI: 5.60 ± 0.44 × 10^{-3} vs. wt/control: 4.90 ± 0.13 × 10^{-3}, ko/cTnI: 6.20 ± 0.52 × 10^{-3} vs. ko/control: 4.86 ± 0.15 × 10^{-3}, $p < 0.05$).

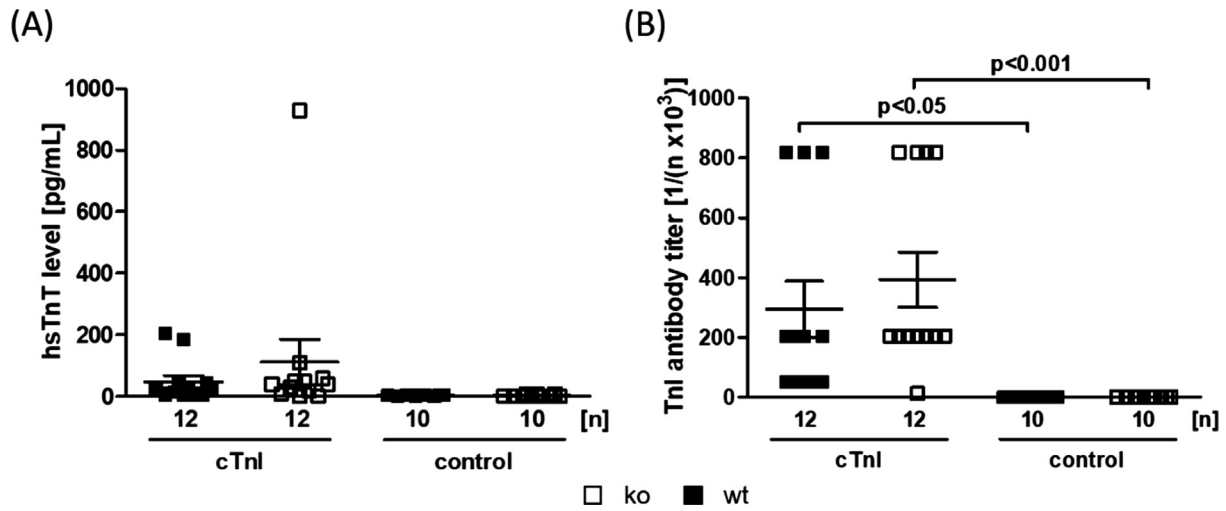

Fig. 5. hsTnT serum level and TnI antibody titer were not altered in adiponectin ko compared to wt mice. Adiponectin ko mice and corresponding wt mice were immunized with cTnI (n = 12) or control buffer (n = 10) and sacrificed on day 28. Determination of hsTnT level (A) and TnI antibody titer (B) in serum. (hsTnT level: wt/cTnI: 48.16 ± 20.13 pg/mL vs. ko/cTnI: 110.83 ± 74.88 pg/mL, wt/control and ko/control < 30 pg/mL; TnI antibody titer: wt/cTnI: $1/(294.40 \pm 93.51 \times 10^{-3})$ vs. ko/cTnI: $1/(393.60 \pm 92.08 \times 10^{-3})$, wt/control and ko/control: $1/(0.8 \times 10^{-3})$).

response, serum cTnI specific IgG (total) autoantibodies were determined 28 days after the initial immunization. Here, the control groups showed no humoral immune response. The mice treated with cTnI showed significantly increased cTnI specific autoantibodies in comparison to the control group ($p < 0.05$). Although the adiponectin ko group showed a slightly higher cTnI autoantibody titer than the wt group, there was no significant difference between the two groups (Fig. 5B).

3.6. Relative mRNA expression of cytokines in the myocardium did not change in adiponectin ko compared to wt mice

To investigate the mRNA expression of several proinflammatory cytokines in the heart, isolated RNA was analyzed in qPCR. The control group showed a low relative mRNA expression of TNFα, IFNγ, IL6 and IL17 with no significant difference between wt and adiponectin ko mice. Mice immunized with cTnI showed a higher expression of these cytokines compared to the control group, with a significant difference in the expression of IFNγ, IL17 and IL6 ($p < 0.05$). The adiponectin ko group treated with cTnI showed a higher expression of IL6 and a lower expression of IL17 compared to the wt/cTnI group. The expression of IFNγ and TNFα was approximately equal in both groups (Fig. 6A–D).

To analyze the influence of an adiponectin ko on the mRNA expression of an antiinflammatory immune response, the relative IL10 mRNA expression was measured. The results showed that both wt and ko mice of the control immunized group displayed a lower IL10 expression compared to the ko and wt animals immunized with cTnI. The difference between these groups was significant only for wt mice. Furthermore, cTnI-immunized wt mice showed a higher expression of IL10 than cTnI-immunized ko mice (Fig. 6E).

3.7. No significant alteration of the relative mRNA expression of adiponectin receptor 1 and 2 could be detected over the course of myocarditis

To better characterize the role of adiponectin in the EAM model, the expression of adiponectin receptor 1 and 2 was assessed during the course of EAM induction via qPCR. The results showed that the relative mRNA expression of adiponectin receptor 1 revealed distinct differences between ko and wt animals. Thus, before the induction of EAM, adiponectin ko mice showed an obviously higher adiponectin receptor 1 expression compared to wt mice. Whereas this expression remained on the same level during the induction of EAM for wt mice, the initially increased cardiac adiponectin receptor 1 expression in adiponectin ko mice obviously decreased during EAM induction regardless of their treatment (Fig. 7A).

In contrast to that, a similar tendency could be detected in all four groups for the relative cardiac mRNA expression of adiponectin receptor 2. During myocarditis induction, a significant decrease in the expression level of this receptor for all groups with no significant difference among the groups could be observed (Fig. 7B). Thus, there is a different impact of the adiponectin ko on the expression of the two adiponectin receptors.

3.8. Relative mRNA expression of C1QTNF1, C1QTNF3, C1QTNF6 and C1QTNF9 in heart tissue was not altered between adiponectin ko and wt mice

We studied the mRNA expression of other C1QTNF family members most suitable for fulfilling the functions of adiponectin in adiponectin ko mice (C1QTNF1, C1QTNF3, C1QTNF6 and C1QTNF9), before the induction of EAM (day 0) and after the manifestation of a myocarditis (day 28). In the time course of the disease, all C1QTNF family members showed the same performance in their expression. No genotype dependent differences of C1QTNF1, C1QTNF3, C1QTNF6 and C1QTNF9 expression could be observed before EAM induction. After the manifestation of a myocarditis, all C1QTNF family members showed a genotype independent increased or steady expression compared to day 0 (Fig. S2). However, after EAM manifestation, the control groups showed a low relative mRNA expression of C1QTNF1, C1QTNF3, C1QTNF6 and C1QTNF9 with no significant difference between wt and adiponectin ko (Fig. 8A–D). Mice immunized with cTnI showed a higher expression of C1QTNF3 and C1QTNF6 compared to the control group, with a significantly higher mRNA expression of C1QTNF3 between adiponectin ko mice immunized with cTnI or control buffer ($p < 0.05$) (Fig. 8B, C). There were no significant differences between wt and adiponectin ko group treated with cTnI (Fig. 8A–D).

4. Discussion

In our study, we investigated the effect of adiponectin deficiency on cardiac parameters in the EAM animal model. Here, we could not demonstrate that adiponectin deficiency triggers a significantly increased impairment of cardiac functions.

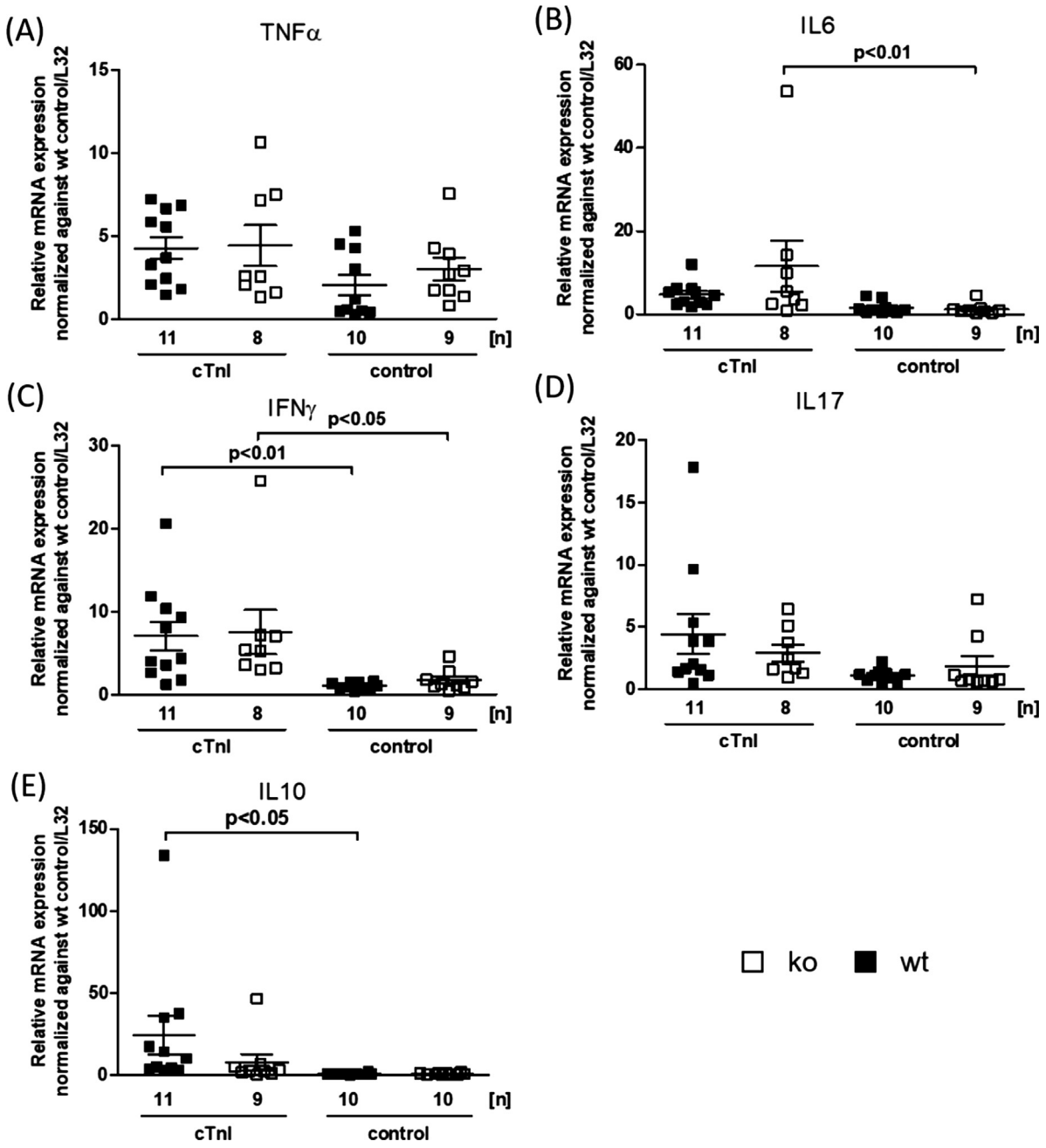

Fig. 6. Relative mRNA expression of pro- and antiinflammatory cytokines in adiponectin ko and wt mice. Adiponectin ko and corresponding wt mice were immunized with cTnI (n = 8–11) or control buffer (n = 9–10), respectively, and sacrificed on day 28. The relative mRNA expression of TNFα, IL6, IFNγ, IL17 ans IL10 was determined. (TNFα: wt/cTnI: 4.27 ± 0.67 vs. ko/cTnI: 4.45 ± 1.24, wt/control: 2.06 ± 0.63 vs. ko/control: 3.01 ± 0.69; IFNγ: wt/cTnI: 7.08 ± 1.74 vs. ko/cTnI: 7.56 ± 2.67, wt/control: 1.08 ± 0.13 vs. ko/control: 1.74 ± 0.42; IL6: wt/cTnI: 4.73 ± 0.88 vs. ko/cTnI: 11.57 ± 6.22, wt/control: 1.55 ± 0.47 vs. ko/control: 1.24 ± 0.43; IL17: wt/cTnI: 4.43 ± 1.56 vs. ko/cTnI: 2.89 ± 0.71, wt/control: 1.10 ± 0.16 vs. ko/control: 1.86 ± 0.78; IL10: wt/cTnI: 24.35 ± 11.58 vs. ko/cTnI: 7.84 ± 4.89, wt/control: 0.98 ± 0.19 vs. ko/control: 0.90 ± 0.14).

Autoimmune myocarditis, which in many cases leads subsequently to DCM, is caused by an overwhelming response of the immune system to body-specific autoantigens. The main molecular mechanisms that are defective in cardiomyopathies are calcium homeostasis in the cell, eNOS expression, apoptosis, and energy metabolism [6,18]. Adiponectin is an adipocytokine that has an antiinflammatory and cardioprotective effect both *in vitro* and in animal experiments [7,9–12]. It also modulates oxidative/nitrate-mediated stress, apoptosis, fibrosis, hypertrophy and cardiac metabolism [6,9]. Thus, adiponectin could influence the pathogenesis of cardiomyopathies.

To initially assess the expression of adiponectin during EAM induction (day 0 and 28), we determined the relative cardiac mRNA expression of adiponectin by qPCR as well as the adiponectin serum level by ELISA during EAM induction. Here, we could observe an increase of adiponectin mRNA expression in cTnI-immunized mice during time course of EAM, whereas control immunized mice showed a steady state adiponectin mRNA expression during this time. These observed results could be based on an increased infiltration rate of CD4 T lymphocytes in hearts of cTnI immunized mice. So, it has already been described that infiltrated CD4 T lymphocyte seems to lead to an increased adiponectin mRNA expression in heart tissue [23]. Due to the fact that hearts of mice suffering from EAM showed also an increased CD4 T lymphocyte infiltration [24], it seems likely that increased cardiac mRNA adiponectin expression directly correlates with an increased

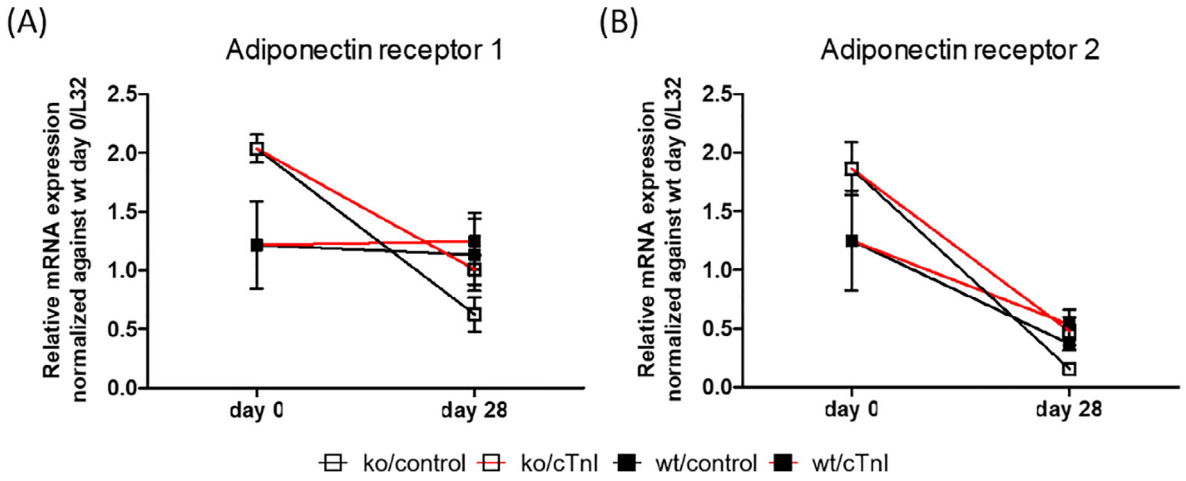

Fig. 7. Relative mRNA expression of adiponectin receptor 1 and 2 during the course of EAM induction. Adiponectin ko and corresponding wt mice were sacrificed on day 0 (n = 5) or 28 (n = 10–16) after the immunization with cTnI or control buffer. Determination of relative mRNA expression of adiponectin receptor 1 (A) and adiponectin receptor 2 (B). (Adiponectin receptor 1: wt/control/day 0: 1.22 ± 0.37 vs. wt/control/day 28: 1.25 ± 0.26; wt/cTnI/day 0: 1.22 ± 0.37 vs. wt/cTnI/day 28: 1.18 ± 0.22; ko/control/day 0: 2.04 ± 0.12 vs. ko/control/day 28: 0.63 ± 0.14; ko/cTnI/day 0: 2.04 ± 0.12 vs. ko/cTnI/day 28: 1.01 ± 0.13; adiponectin receptor 2: wt/control/day 0: 1.25 ± 0.43 vs. wt/control/day 28: 0.37 ± 0.04; wt/cTnI/day 0: 1.25 ± 0.43 vs. wt/cTnI/day 28: 0.53 ± 0.11; ko/control/day 0: 1.87 ± 0.23 vs. ko/control/day 28: 0.16 ± 0.03; ko/cTnI/day 0: 1.87 ± 0.23 vs. ko/cTnI/day 28: 0.48 ± 0.12).

CD4 T lymphocyte infiltrate in cTnI-immunized mice.

In contrast to that, a decrease of serum level from day 0 to day 28 could be detected for cTnI as well as control immunized mice. These results are in line with the findings in other animal models, where such a decrease of adiponectin expression could be ascribed to the increasing age of the animals [25]. Thus, the time-dependent reduction of adiponectin serum level might be an age depending effect.

Moreover, a detailed consideration of cardiac adiponectin mRNA at day 28 showed that cTnI-immunized mice had a significantly increased mRNA expression compared to control treated mice. This observation is in accordance with already published results focusing on the adiponectin expression level of various autoimmune diseases. So, adiponectin has been found to be increased in patients suffering from type I diabetes, ankylosing spondylitis, systemic lupus erythematosus and ulcerative colitis [21,26–28].

Apart from the significant difference in the cardiac mRNA level of adiponectin observed between cTnI and control immunized mice, no immunization-dependent variations of the adiponectin serum levels could be measured. Such variations between heart and serum adiponectin level were already described for DCM patients. Here, the cardiac adiponectin protein expression is down-regulated in DCM patients compared to control, whereas the serum adiponectin level of both groups showed no difference [26].

Based on the significantly increased cardiac mRNA expression level of adiponectin in cTnI-immunized compared to control-immunized mice and despite of missing alterations of serum adiponectin level in these groups, we decided to analyze the effect of adiponectin on the induction of an autoimmune myocarditis. Here, we induced an EAM in adiponectin ko as well as corresponding wt mice by immunization with cTnI. In accordance with our previous studies, the mice developed an autoimmune myocarditis with a significant deterioration of cardiac parameters compared to the control group [29–31]. Compared to the wt group, the adiponectin ko group immunized with cTnI showed no significant difference concerning the inflammation and fibrosis. However, in order to better understand the effect of an adiponectin ko on the infiltrated leucocyte population, we also determined the type and amount of the recruited leucocyte population into the myocardium. There was no significant difference in the infiltration pattern of CD4 and CD8 T lymphocytes, B lymphocytes and macrophages in cTnI-immunized adiponectin ko mice compared to the wt group. Furthermore, in contrast to CD4, CD8 T lymphocytes seems not playing an important role in the induction of an EAM. Only a small amount of CD8 T lymphocytes infiltrated the myocardium in adiponectin ko and wt mice, whereas the amount of infiltrated CD4 T lymphocytes was very high compared to all other cell types. Similar results could already be observed for EAM mice. Here, CD4 T lymphocytes seem to be the predominate leukocyte population in the myocardial infiltrate in acute EAM. This predominance of CD4 T lymphocytes might be based on their requirement for an initiation of a myocarditis. In contrast to that, other leukocyte populations like CD8 infiltrate the myocardium to a subsequent date, able to directly affect cardiomyocyte function. These leukocyte populations (CD8, CD45 and CD68) seem not to be involved in the induction of an EAM [24]. Thus, the high amount of infiltrated CD4 T lymphocytes coming along with a very small CD8 T lymphocyte infiltrate can be ascribed to the early phase of myocarditis manifestation analyzed in this study. However, this state-dependent infiltration pattern of the myocardium might not be influenced by adiponectin expression.

There are several potential explanations why adiponectin deficiency does not have significant effects on the severity of inflammation in the murine EAM model.

First, different mouse strains react differently to gene silencing. It is also known that the susceptibility to the development of inflammation in mice with different background is variable. Parker *et al.* showed that among adiponectin ko mice on a C57BL/6, MRL or MRL-lpr background, only mice on the MRL-lpr background were able to develop an autoimmune phenotype of lupus. Compared to wt littermates, these mice had a greater lymphadenopathy and splenomegaly, kidney alteration, increased anti-nuclear antibody and anti-double stranded DNA production [32]. To our knowledge, this is the first study to investigate the effects of adiponectin deficiency in mice on A/J background.

Second, even while using animals on the same background, a broad range of endpoints could be demonstrated in adiponectin ko mice compared to the wt littermates [33]. Jin *et al.* studied the role of adiponectin in ischemic acute kidney injury. Compared to wt mice, C57BL/6 adiponectin ko mice had lower serum creatinine, less tubular damage or apoptosis, reduced activation of p53 and caspase-3, less infiltration of neutrophils, macrophages, and T lymphocytes, reduced expression of the proinflammatory molecules IL6, TNFα, monocyte chemoattractant protein-1, and macrophage inflammatory protein-2 [34]. Apart from Jin *et al.,* Pini *et al.* studied the role of adiponectin in the development of spontaneous colitis in IL10 ko mice. IL10/adiponectin ko mice on a

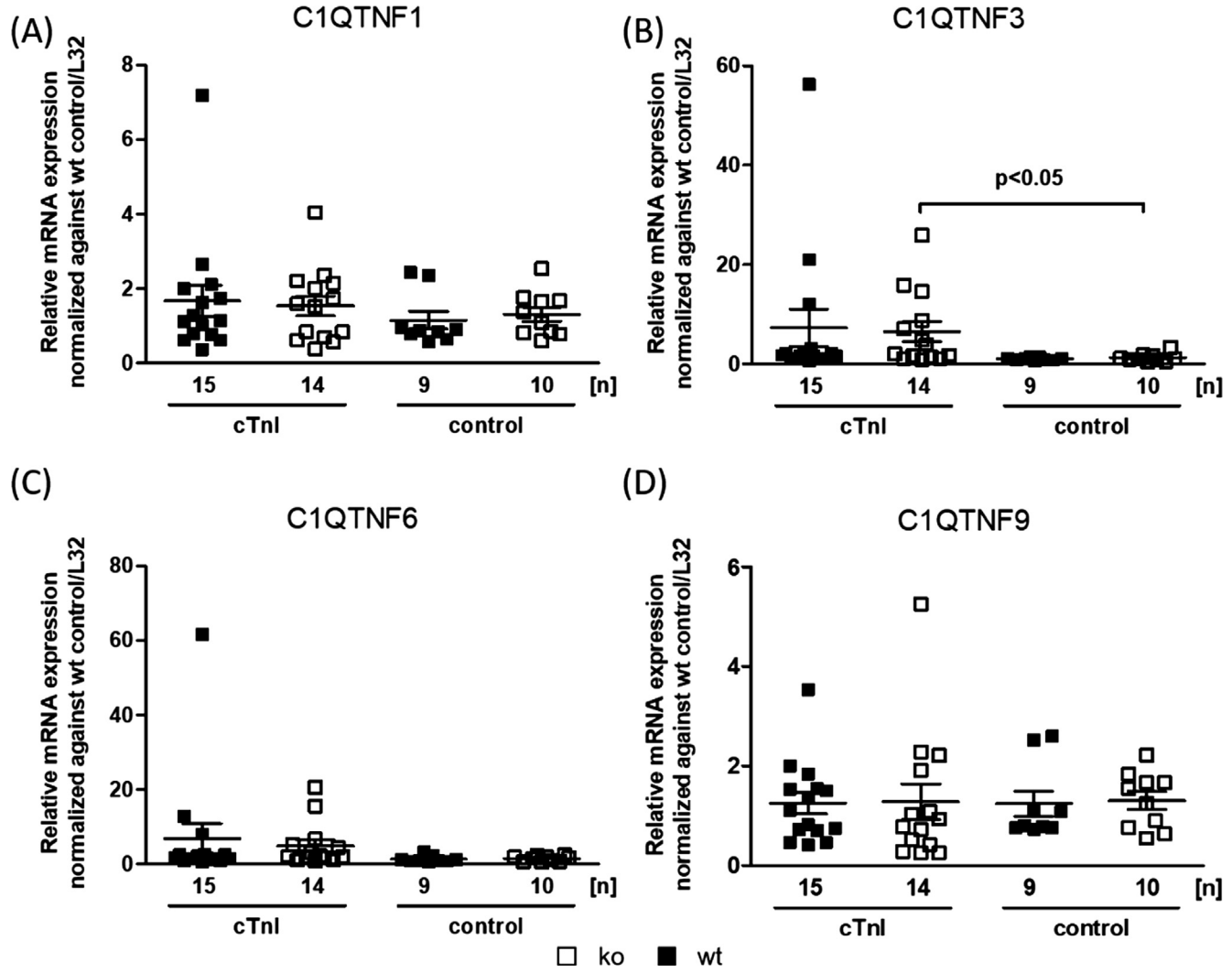

Fig. 8. Relative mRNA expression of C1QTNF family members revealed no difference between adiponectin ko and wt mice. Adiponectin ko (n = 10–14) and corresponding wt mice (n = 9–15) were immunized with cTnI or control buffer and sacrificed on day 28. Determination of relative mRNA expression of C1QTNF1 (A), C1QTNF3 (B), C1QTNF6 (C) and C1QTNF9 (D). (C1QTNF1: wt/cTnI 1.66 ± 0.43 vs. wt/control: 1.15 ± 0.24; ko/cTnI: 1.53 ± 0.27 vs. ko/control: 1,0.1 ± 0.19; C1QTNF3: wt/cTnI: 7.25 ± 3.78 vs. wt/control: 1.01 ± 0.11; ko/cTnI: 6.47 ± 2.00 vs. ko/control: 1.26 ± 0.27, $p < 0.05$; C1QTNF6: wt/cTnI: 6.72 ± 4.01 vs. wt/control: 1.26 ± 0.30; ko/cTnI: 4.82 ± 1.59 vs. ko/control: 1.42 ± 0.22; C1QTNF9: wt/cTnI: 1.25 ± 0.21 vs. wt/control: 1.24 ± 0.25; ko/cTnI: 1.28 ± 0.36 vs. ko/control: 1.31 ± 0.18).

C57BL/6J background showed no significant differences in inflammatory infiltrate or crypt elongation, circulating levels of serum amyloid A and IFNγ, body weight progression and bone mass to that of single IL10 ko mice [35]. Furthermore, on one hand, Fayad *et al.* showed that adiponectin ko mice on a C57BL/6J background were protected from chemically induced colitis. This could be determined with clinical and histologic scores, assessment of the proliferation of epithelial cells and measurement of cytokines and basic fibroblast growth factor expression [36]. On the other hand, Nishihara *et al.* found, that adiponectin ko mice on a C57BL/6J background developed much more severe chemically induced colitis compared with wt mice, followed by higher mRNA expression levels of chemokines and increased cellular infiltration, including macrophages [37].

To better understand the effect of adiponectin deficiency on cardiac parameters in the EAM, we measured hsTnT serum concentration, which demonstrates the severity of cardiomyocytes damage. Furthermore, we determined the heart-to-body-weight ratio and the weight gain in all mice. Compared to wt group, the adiponectin ko group immunized with cTnI showed a tendency towards increased hsTnT serum concentration and heart-to-body-weight ratio accompanied by a decreased weight gain, pointing to the heart enlargement in course of myocarditis. However, the differences to the wt group treated with cTnI were not significant. We would like to point out, that the absence of significant differences between the adiponectin ko and wt groups is not due to a faulty induction of EAM. In this experiment, mice immunized with cTnI developed a significant myocardial inflammation and fibrosis. They also showed less body mass progression, higher heart-to-body-weight ratio and higher hsTnT serum level. The mice treated with cTnI had significantly increased cTnI specific autoantibodies and a higher expression of the proinflammatory cytokines (TNFα, IFNγ, IL6 and IL17) compared to the control group, with a significant difference in the expression of IFNγ, IL17 and IL6.

Due to the fact that many research groups found that adiponectin has an effect on pro- and antiinflammatory cytokines like IL6, IL17 and IFNγ [6–9,11,18–20,38], as a next step pro- as well as antiinflammatory cytokines were analyzed to further characterize the effect of an adiponectin deficiency. Nonetheless, we could not demonstrate an adiponectin-dependent regulation of proinflammatory cytokines in our experiment. So, there was no significant difference in the mRNA expression of TNFα, IFNγ, IL6 and IL17 in the heart tissue of adiponectin ko and wt mice. Only a tendency towards increased mRNA expression of IL6 could be determined in adiponectin ko group.

Acknowledging the importance of the antiinflammatory cytokine IL10 in the course of myocarditis as shown by Izumi *et al.* and supported through the findings of Watanabe *et al.* [39,40], we determined the relative mRNA expression of IL10 in the cardiac tissue in adiponectin ko and wt mice. We were able to show that immunization with cTnI caused an increase in the relative mRNA expression of IL10 in both ko and wt groups compared to the treatment with control buffer with a significant difference between the two wt groups.

We also measured a lower mRNA expression of IL10 in ko animals compared to corresponding wt mice. However, this difference was not significant. Thus, an adiponectin deficiency seems to lead to a decreased synthesis of IL10. However, this effect is not sufficient to induce a significantly impaired cardiac function of cTnI-immunized adiponectin ko mice compared to wt mice also immunized with cTnI. It cannot be ruled out that the expression of IL10 may be partly compensated by other immunological pathways like one of PPARα in the absence of adiponectin [41].

To identify further reasons why adiponectin ko mice did not show differences in the disease progression of an EAM, we investigated possible compensatory mechanisms of the adiponectin deficiency. First, we determined the relative mRNA expression of adiponectin receptors 1 and 2 in cardiac tissue. Here, a different impact of the adiponectin ko on the expression of the two adiponectin receptors could be observed. This difference could be based on the primary location of both receptors. Whereas adiponectin receptor 1 is predominantly expressed in the musculature [5], adiponectin receptor 2 is mainly expressed in the liver [5]. Thus, a strict regulation of adiponectin receptor 1 might be more important to maintain cardio protective effects of adiponectin than adiponectin receptor 2, which seems to play a secondary role in the EAM model. This hypothesis would explain why adiponectin receptor 1 showed an immunization-independent steady mRNA expression level in wt mice, whereas the mRNA level of adiponectin receptor 2 decreased for cTnI as well as control immunized mice.

In addition to that, our results indicated that at least the strict regulation of adiponectin receptor 1 seems to be adiponectin-dependent. Accordingly, adiponectin ko mice showed a time-dependent decrease of adiponectin receptor 1 expression, what could be ascribed to the missing stimulation by its ligand. Such a feedback mechanism between adiponectin and adiponectin receptor 1 was already observed. So, Li *et al.* showed that adiponectin receptor 1 positively correlates with myocardial adiponectin [42]. For adiponectin receptor 2, the decreased mRNA expression in cTnI and control immunized ko mice could be ascribed to other regulatory effects as the expression level of adiponectin receptor 2 of immunized wt mice behaved the same. Thus, an adiponectin-dependent feedback mechanism would explain only the time-dependent decrease of adiponectin receptor 1 mRNA expression in adiponectin ko mice. However, detailed mechanisms of adiponectin receptor 1 and 2 expression regulation and the connection to the induction of an EAM have to be elucidated in future experiments.

Another possible explanation why adiponectin deficiency does not have significant effects on the severity of murine EAM is the existence of other ligands of the adiponectin receptors, which bind to the receptor in the absence of adiponectin and activate compensatory signal cascades. It is known that the C1QTNF family, where adiponectin belongs to, has a variety of members with high amino acid sequence homology [13]. Accordingly, it cannot be ruled out that the above-mentioned C1QTNF members may partially or even completely compensate the functions of adiponectin in its absence [43]. Thus, we studied the mRNA expression of the C1QTNF family members, most suitable for fulfilling the functions of adiponectin in adiponectin ko mice (C1QTNF1, C1QTNF3, C1QTNF6 and C1QTNF9) in EAM model in the course of the disease. Here, both ko and wt animals showed concordant changes in the expression of all analyzed C1QTNF members during myocarditis. Furthermore, we were not able to show any significant difference in the upregulation of the mRNA expression of these C1QTNF family members in the heart tissue between adiponectin ko and corresponding wt mice immunized with cTnI. Therefore, it is unlikely that the absence of adiponectin could be compensated by other C1QTNF family members in the murine model of cTnI-induced EAM. Nevertheless, this conclusion is only drawn on the basis of mRNA expression analyses. Due to translational variations, the protein expression of these ligands could significantly differ from the observed mRNA expression. However, C1QTNF family members seems to be unaffected by such variations. Thus, Wu *et al.* observed for C1QTNF1 a direct correlation between cardiac C1QTNF1 mRNA and protein expression in hypertrophic mice [44]. Like already described by other groups, hypertrophy seems to play a role in myocarditis as well [45]. The increased heart-to-body-weight ratio of cTnI compared to control immunized mice, which we measured in our experiments, supports this hypothesis. Thus, a direct correlation of the mRNA and protein expression level of C1QTNF1 and probably also C1QTNF3, C1QTNF6 and C1QTNF9 in EAM mouse hearts is likely. Despite this correlation between cardiac mRNA and protein expression, the analyzed C1QTNF family members seem not to be responsible for the compensatory effect of adiponectin. Thus, other synergistic effects or alternative signal cascades e.g. signaling via T-cadherin, which mediate antiinflammatory action, might play a role [46–48]. However, these signaling cascades have to be investigated in future studies to understand the role and function of adiponectin in autoimmune myocarditis.

Taken together, although adiponectin is well-known for its antiinflammatory and cardioprotective effects both *in vitro* and in animal experiments, adiponectin deficiency does not lead to a significantly increased impairment of cardiac function in the mouse model of cTnI-induced EAM.

5. Conclusions

Although adiponectin is well known for its cardioprotective, antiinflammatory and immunomodulatory effects, its role in various *in vivo* models of inflammatory diseases is controversial. In many studies, both positive and negative, but also no effects of the adiponectin deficiency on the endpoint results could be shown. In our study, we were able to observe a significant increase in cardiac mRNA expression of adiponectin in mice with EAM. However we could not demonstrate that adiponectin deficiency leads to a significantly increased impairment of cardiac function. Furthermore, it is also unlikely that the absence of adiponectin could be compensated by its receptors or other C1QTNF family members in the murine model of cTnI-induced EAM. Here, other synergistic or redundant effects might play a role and have to be investigated in further studies to understand the role and function of adiponectin in autoimmune myocarditis.

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Declaration of interest

None.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2018.12.022.

References

[1] F. Leuschner, H.A. Katus, Z. Kaya, Autoimmune myocarditis: past, present and future, J. Autoimmunity 33 (3–4) (2009) 282–289.
[2] A.M. Feldman, D. McNamara, Myocarditis, N. Engl. J. Med. 343 (19) (2000) 1388–1398.
[3] O. Zimmermann, M. Kochs, T.P. Zwaka, Z. Kaya, P.M. Lepper, M. Bienek-Ziolkowski, M. Hoher, V. Hombach, J. Torzewski, Myocardial biopsy based classification and treatment in patients with dilated cardiomyopathy, Int. J. Cardiol. 104 (1) (2005) 92–100.
[4] T. Takahashi, S. Saegusa, H. Sumino, T. Nakahashi, K. Iwai, S. Morimoto, T. Kanda, Adiponectin replacement therapy attenuates myocardial damage in leptin-deficient mice with viral myocarditis, J. Int. Med. Res. 33 (2) (2005) 207–214.
[5] T. Yamauchi, J. Kamon, Y. Ito, A. Tsuchida, T. Yokomizo, S. Kita, T. Sugiyama, M. Miyagishi, K. Hara, M. Tsunoda, K. Murakami, T. Ohteki, S. Uchida, S. Takekawa, H. Waki, N.H. Tsuno, Y. Shibata, Y. Terauchi, P. Froguel, K. Tobe, S. Koyasu, K. Taira, T. Kitamura, T. Shimizu, R. Nagai, T. Kadowaki, Cloning of adiponectin receptors that mediate antidiabetic metabolic effects, Nature 423 (6941) (2003) 762–769.
[6] C. Skurk, F. Wittchen, L. Suckau, H. Witt, M. Noutsias, H. Fechner, H.P. Schultheiss, W. Poller, Description of a local cardiac adiponectin system and its deregulation in dilated cardiomyopathy, Eur. Heart J. 29 (9) (2008) 1168–1180.
[7] T. Pischon, C.J. Girman, G.S. Hotamisligil, N. Rifai, F.B. Hu, E.B. Rimm, Plasma adiponectin levels and risk of myocardial infarction in men, JAMA 291 (14) (2004) 1730–1737.
[8] P. Bobbert, C. Scheibenbogen, A. Jenke, G. Kania, S. Wilk, S. Krohn, J. Stehr, U. Kuehl, U. Rauch, U. Eriksson, H.P. Schultheiss, W. Poller, C. Skurk, Adiponectin expression in patients with inflammatory cardiomyopathy indicates favourable outcome and inflammation control, Eur. Heart J. 32 (9) (2011) 1134–1147.
[9] C. Caselli, A. D'Amico, M. Cabiati, T. Prescimone, S. Del Ry, D. Giannessi, Back to the heart: the protective role of adiponectin, Pharmacol Res 82 (2014) 9–20.
[10] G. Witberg, C.R. Ayers, A.T. Turer, E. Lev, R. Kornowski, J. de Lemos, I.J. Neeland, Relation of adiponectin to all-cause mortality, cardiovascular mortality, and major adverse cardiovascular events (from the Dallas Heart Study), Am. J. Cardiol. 117 (4) (2016) 574–579.
[11] N.J. Tomicek, J.C. Hunter, A.M. Machikas, V. Lopez, D.H. Korzick, Acute adiponectin delivery is cardioprotective in the aged female rat heart, Geriatr. Gerontol. Int. 15 (5) (2015) 636–646.
[12] Z.J. Wu, Y.J. Cheng, W.J. Gu, L.H. Aung, Adiponectin is associated with increased mortality in patients with already established cardiovascular disease: a systematic review and meta-analysis, Metabolism 63 (9) (2014) 1157–1166.
[13] A. Schaffler, C. Buechler, CTRP family: linking immunity to metabolism, Trends Endocrinol. Metab. 23 (4) (2012) 194–204.
[14] Q. Zheng, Y. Yuan, W. Yi, W.B. Lau, Y. Wang, X. Wang, Y. Sun, B.L. Lopez, T.A. Christopher, J.M. Peterson, G.W. Wong, S. Yu, D. Yi, X.L. Ma, C1q/TNF-related proteins, a family of novel adipokines, induce vascular relaxation through the adiponectin receptor-1/AMPK/eNOS/nitric oxide signaling pathway, Arterioscler Thromb. Vasc. Biol. 31 (11) (2011) 2616–2623.
[15] M.J. Kim, W. Lee, E.J. Park, S.Y. Park, C1qTNF-related protein-6 increases the expression of interleukin-10 in macrophages, Mol. Cells 30 (1) (2010) 59–64.
[16] A. Kopp, M. Bala, C. Buechler, W. Falk, P. Gross, M. Neumeier, J. Scholmerich, A. Schaffler, C1q/TNF-related protein-3 represents a novel and endogenous lipopolysaccharide antagonist of the adipose tissue, Endocrinology 151 (11) (2010) 5267–5278.
[17] S.A. Compton, B. Cheatham, CTRP-3: blocking a toll booth to obesity-related inflammation, Endocrinology 151 (11) (2010) 5095–5097.
[18] F. Wittchen, L. Suckau, H. Witt, C. Skurk, D. Lassner, H. Fechner, I. Sipo, U. Ungethum, P. Ruiz, M. Pauschinger, C. Tschope, U. Rauch, U. Kuhl, H.P. Schultheiss, W. Poller, Genomic expression profiling of human inflammatory cardiomyopathy (DCMi) suggests novel therapeutic targets, J. Mol. Med. (Berl) 85 (3) (2007) 257–271.
[19] A. Jenke, S. Wilk, W. Poller, U. Eriksson, A. Valaperti, B.H. Rauch, A. Stroux, P. Liu, H.P. Schultheiss, C. Scheibenbogen, C. Skurk, Adiponectin protects against Toll-like receptor 4-mediated cardiac inflammation and injury, Cardiovasc. Res. 99 (3) (2013) 422–431.
[20] X.Q. Tian, Y.J. Yang, Q. Li, P.S. Huang, X.D. Li, C. Jin, K. Qi, L.P. Jiang, G.H. Chen, Globular adiponectin inhibits the apoptosis of mesenchymal stem cells induced by hypoxia and serum deprivation via the AdipoR1-mediated pathway, Cell Physiol. Biochem. 38 (3) (2016) 909–925.
[21] K.E. Sada, Y. Yamasaki, M. Maruyama, H. Sugiyama, M. Yamamura, Y. Maeshima, H. Makino, Altered levels of adipocytokines in association with insulin resistance in patients with systemic lupus erythematosus, J. Rheumatol. 33 (8) (2006) 1545–1552.
[22] M. Andrassy, H.C. Volz, B. Maack, A. Schuessler, G. Gitsioudis, N. Hofmann, D. Laohachewin, A.R. Wienbrandt, Z. Kaya, A. Bierhaus, E. Giannitsis, H.A. Katus, G. Korosoglou, HMGB1 is associated with atherosclerotic plaque composition and burden in patients with stable coronary artery disease, PloS one 7 (12) (2012) e52081.
[23] S. Danturti, K.S. Keslar, L.R. Steinhoff, R. Fan, N. Dvorina, A. Valujskikh, R.L. Fairchild, W.M. Baldwin 3rd, CD4+ T lymphocytes produce adiponectin in response to transplants, JCI Insight 2 (12) (2017).
[24] M. Afanasyeva, D. Georgakopoulos, N.R. Rose, Autoimmune myocarditis: cellular mediators of cardiac dysfunction, Autoimmun. Rev. 3 (7–8) (2004) 476–486.
[25] J.M. Muller-Delp, K. Hotta, B. Chen, B.J. Behnke, J.J. Maraj, M.D. Delp, T.R. Lucero, J.A. Bramy, D.B. Alarcon, H.E. Morgan, M.R. Cowan, A.D. Haynes, Effects of age and exercise training on coronary microvascular smooth muscle phenotype and function, J. Appl. Physiol. (1985) 124 (1) (2018) 140–149.
[26] C.S. Derdemezis, T.D. Filippatos, P.V. Voulgari, A.D. Tselepis, A.A. Drosos, D.N. Kiortsis, Leptin and adiponectin levels in patients with ankylosing spondylitis. The effect of infliximab treatment, Clin. Exp. Rheumatol. 28 (6) (2010) 880–883.
[27] A. Imagawa, T. Funahashi, T. Nakamura, M. Moriwaki, S. Tanaka, H. Nishizawa, K. Sayama, S. Uno, H. Iwahashi, K. Yamagata, J. Miyagawa, Y. Matsuzawa, Elevated serum concentration of adipose-derived factor, adiponectin, in patients with type 1 diabetes, Diabetes Care 25 (9) (2002) 1665–1666.
[28] J. Weigert, F. Obermeier, M. Neumeier, J. Wanninger, M. Filarsky, S. Bauer, C. Aslanidis, G. Rogler, C. Ott, A. Schaffler, J. Scholmerich, C. Buechler, Circulating levels of chemerin and adiponectin are higher in ulcerative colitis and chemerin is elevated in Crohn's disease, Inflamm. Bowel Dis. 16 (4) (2010) 630–637.
[29] Z. Kaya, S. Goser, S.J. Buss, F. Leuschner, R. Ottl, J. Li, M. Volkers, S. Zittrich, G. Pfitzer, N.R. Rose, H.A. Katus, Identification of cardiac troponin I sequence motifs leading to heart failure by induction of myocardial inflammation and fibrosis, Circulation 118 (20) (2008) 2063–2072.
[30] Z. Kaya, H.A. Katus, N.R. Rose, Cardiac troponins and autoimmunity: their role in the pathogenesis of myocarditis and of heart failure, Clin. Immunol. 134 (1) (2010) 80–88.
[31] A.M. Muller, A. Fischer, H.A. Katus, Z. Kaya, Mouse models of autoimmune diseases - autoimmune myocarditis, Curr. Pharm. Des. 21 (18) (2015) 2498–2512.
[32] J. Parker, H. Menn-Josephy, B. Laskow, Y. Takemura, T. Aprahamian, Modulation of lupus phenotype by adiponectin deficiency in autoimmune mouse models, J. Clin. Immunol. 31 (2) (2011) 167–173.
[33] G. Fantuzzi, Adiponectin in inflammatory and immune-mediated diseases, Cytokine 64 (1) (2013) 1–10.
[34] X. Jin, J. Chen, Z. Hu, L. Chan, Y. Wang, Genetic deficiency of adiponectin protects against acute kidney injury, Kidney Int. 83 (4) (2013) 604–614.
[35] M.E. Gove, M. Pini, R. Fayad, R.J. Cabay, G. Fantuzzi, Adiponectin deficiency modulates adhesion molecules expression and cytokine production but does not affect disease severity in the transfer model of colitis, Cytokine 47 (2) (2009) 119–125.
[36] R. Fayad, M. Pini, J.A. Sennello, R.J. Cabay, L. Chan, A. Xu, G. Fantuzzi, Adiponectin deficiency protects mice from chemically induced colonic inflammation, Gastroenterology 132 (2) (2007) 601–614.
[37] T. Nishihara, M. Matsuda, H. Araki, K. Oshima, S. Kihara, T. Funahashi, I. Shimomura, Effect of adiponectin on murine colitis induced by dextran sulfate sodium, Gastroenterology 131 (3) (2006) 853–861.
[38] Y. Liao, S. Takashima, N. Maeda, N. Ouchi, K. Komamura, I. Shimomura, M. Hori, Y. Matsuzawa, T. Funahashi, M. Kitakaze, Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism, Cardiovasc. Res. 67 (4) (2005) 705–713.
[39] T. Izumi, H. Takehana, C. Matsuda, H. Yokoyama, K. Kohno, K. Suzuki, T. Inomata, Experimental autoimmune myocarditis and its pathomechanism, Herz 25 (3) (2000) 274–278.
[40] K. Watanabe, M. Nakazawa, K. Fuse, H. Hanawa, M. Kodama, Y. Aizawa, T. Ohnuki, F. Gejyo, H. Maruyama, J. Miyazaki, Protection against autoimmune myocarditis by gene transfer of interleukin-10 by electroporation, Circulation 104 (10) (2001) 1098–1100.
[41] W. Gao, W. Xu, H. Li, The expression and role of peroxisome proliferator-activated receptor alpha in atherosclerosis, Mol. Med. Rep. 7 (3) (2013) 862–868.
[42] J. Li, S. Su, X. Zong, Analysis of the association between adiponectin, adiponectin receptor 1 and diabetic cardiomyopathy, Exp. Ther. Med. 7 (4) (2014) 1023–1027.
[43] M. Braun, N. Hettinger, C. Koentges, K. Pfeil, M.C. Cimolai, M.M. Hoffmann, M. Osterholt, T. Doenst, C. Bode, H. Bugger, Myocardial mitochondrial and contractile function are preserved in mice lacking adiponectin, PLoS One 10 (3) (2015) e0119416.
[44] L. Wu, L. Gao, D. Zhang, R. Yao, Z. Huang, B. Du, Z. Wang, L. Xiao, P. Li, Y. Li, C. Liang, Y. Zhang, C1QTNF1 attenuates angiotensin II-induced cardiac hypertrophy via activation of the AMPKa pathway, Free Radic. Biol. Med. 121 (2018) 215–230.
[45] A. Matsumori, C. Kawai, An animal model of congestive (dilated) cardiomyopathy: dilatation and hypertrophy of the heart in the chronic stage in DBA/2 mice with myocarditis caused by encephalomyocarditis virus, Circulation 66 (2) (1982) 355–360.
[46] M.S. Denzel, M.C. Scimia, P.M. Zumstein, K. Walsh, P. Ruiz-Lozano, B. Ranscht, T-cadherin is critical for adiponectin-mediated cardioprotection in mice, J. Clin. Invest. 120 (12) (2010) 4342–4352.
[47] J.L. Parker-Duffen, K. Nakamura, M. Silver, R. Kikuchi, U. Tigges, S. Yoshida, M.S. Denzel, B. Ranscht, K. Walsh, T-cadherin is essential for adiponectin-mediated revascularization, J. Biol. Chem. 288 (34) (2013) 24886–24897.
[48] C. Hug, J. Wang, N.S. Ahmad, J.S. Bogan, T.S. Tsao, H.F. Lodish, T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin, Proc. Natl. Acad. Sci. USA 101 (28) (2004) 10308–10313.