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Modulation of Lupus Phenotype by Adiponectin Deficiency in Autoimmune Mouse Models

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Abstract

Adiponectin is an adipocyte-derived cytokine with anti-inflammatory properties. Paradoxically, circulating adiponectin levels are increased in a number of inflammatory diseases. Thus, we sought to define the role of adiponectin deficiency in mouse models of autoimmunity. Adiponectin-deficient mice on a C57BL/6 background do not develop an autoimmune phenotype. Autoimmunity was also not observed in adiponectin-deficient mice generated on the permissive MRL background. However, adiponectin deficiency exacerbated the autoimmune phenotype of MRL-*lpr* mice. Compared with MRL-*lpr* mice, MRL-*lpr.apn*^{-/-} mice displayed greater lymphadenopathy and splenomegaly, as well as increased anti-nuclear antibody and anti-dsDNA production. In addition, evaluation of the kidney revealed larger glomerular tuft size, crescent formation, increased IgG and C3 deposits, and mesangial expansion in the MRL-*lpr.apn*^{-/-} mice. The effects of adiponectin deficiency on the autoimmune phenotypes were more pronounced in female versus male mice. These data show that, while adiponectin deficiency is not sufficient to confer autoimmunity, adiponectin acts as a negative modulator of the autoimmune phenotype in a murine model of lupus.

Keywords

Adiponectin; lupus; mouse model; inflammation; autoimmunity

Introduction

Adiponectin is a cytokine specifically produced by adipose tissue that has a protective role in various metabolic and cardiovascular diseases [1, 2]. Adiponectin is abundantly present in serum and represents 0.01% of the total serum protein. Lean, healthy individuals typically express the highest circulating levels of adiponectin, and levels decline as body mass increases [3, 4]. Presumably, this down-regulation occurs because the proinflammatory cytokines TNF α and IL-6, which are upregulated in obese states, inhibit the synthesis of adiponectin by adipocytes [5–7]. Serum adiponectin levels are significantly higher in women compared with men [8]. A low adiponectin level is associated with the metabolic syndrome and the development of type 2 diabetes [9]. Low serum adiponectin is also a risk factor for a number of cardiovascular disorders [10, 11]. With regard to age, adiponectin levels in men increase linearly with age, and levels in women increase dramatically until age 50 [8]. In addition, elevated serum levels of adiponectin are observed in centenarians and their offspring, and this was also associated with polymorphisms in the adiponectin gene which result in higher adiponectin levels [12]. Thus, it has been proposed that high levels of adiponectin may be beneficial towards increasing life span.

The protective actions of adiponectin in metabolic and cardiovascular diseases have been elucidated by studies of mice that lack a functional *adiponectin* gene. Adiponectin-deficient mice are viable and they breed normally. However, mice lacking adiponectin exhibit greater insulin resistance when placed on a high caloric diet [13] and they develop hypertension when placed on a high-salt diet [14]. Adiponectin-deficient mice also display larger atherosclerotic lesions, increased accumulation of T lymphocytes within lesions and increased plasma levels of interferon-inducible protein 10 when they are bred to atherosclerosis prone apoE^{-/-} mice [15, 16]. Adiponectin-deficient mice are more prone to injury in surgical models of myocardial ischemia-reperfusion injury, myocardial infarction, hypertrophic cardiomyopathy, and peripheral artery disease [17–20].

It is widely recognized that adiponectin has anti-inflammatory properties. In patient populations, serum adiponectin levels inversely correlate with the pro-inflammatory marker proteins C-reactive protein and IL-6 [21–24]. In addition, TNF α levels are elevated in adiponectin-deficient mice; and in cell culture experiments, it can be shown that adiponectin diminishes LPS-stimulated TNF α release [20, 25].

While it is generally accepted that adiponectin has anti-inflammatory properties, it is paradoxical that patients with autoimmune disease who exhibit elevated levels of this protein [26]. For example, despite high levels of TNF α , which would normally serve to inhibit adiponectin production, systemic lupus erythematosus (SLE) patients have higher adiponectin levels compared with controls [27]. Increased levels of adiponectin have also been observed in the synovial fluid of patients with rheumatoid arthritis [28]. Further, it has been shown that patients with type 1 diabetes have increased levels of adiponectin whereas a decrease is observed in patients with type 2 diabetes [29, 30]. Thus, the role of adiponectin in autoimmunity requires further study. To address this issue, we examined the consequences of adiponectin deficiency on the phenotypes associated with autoimmunity in C57BL/6, MRL, and MRL-*lpr* strains of mice.

Materials and Methods

Animals

Eight-week-old MRL and MRL-*lpr* mice were purchased from Jackson Laboratories. MRL-*apn*^{-/-} mice were generated by backcrossing C57BL/6.*apn*^{-/-} mice to MRL/MpJ strain for six generations. MRL-*lpr.apn*^{-/-} mice were generated by crossing MRL-*apn*^{-/-} mice to MRL-*lpr* mice to generate mice heterozygous for both mutations. These heterozygous mice were intercrossed to obtain the mice used for this study. Study protocols were approved by the Institutional Animal Care and Use Committee at Boston University School of Medicine.

Study Protocol

Mice were maintained on a normal mouse chow and sacrificed at 8 or 20 weeks of age. Mice were weighed and blood was drawn by cardiac puncture. Skin was retracted from the chest to under the mouth in order to expose the submandibular lymph nodes. From each mouse, all superficial cervical lymph nodes were excised and weighed wet to calculate weight. Spleen was also excised and weighed wet. Kidneys from each mouse were immediately fixed in OCT for frozen sectioning or 10% neutral-buffered formalin overnight and processed for embedding in paraffin.

Kidney Histology

Paraffin-embedded tissue was sectioned (5 μ thick) and slides were stained with hematoxylin and eosin. Cross-sectional areas of at least 25 glomeruli were measured in each animal using computer-assisted pixel counting (Photoshop CS3; Adobe). Frozen tissue was sectioned and immune complex deposition in glomeruli was determined by histological staining of frozen sections with Cy3-conjugated anti-mouse IgG(Fc)F(ab')₂ (Sigma) or fluorescein isothiocyanate (FITC)-conjugated anti-mouse C3 (Cappel). Slides were examined by two blinded investigators.

Serological Assays

Circulating anti-nuclear antibody (ANA) levels were measured by immunofluorescence using HEp-2 coated slides (The Binding Site Inc., San Diego, CA, USA). Slides were incubated for 1 h with serial log-scale dilutions (1:100–1:90,000; as previously described by Komori et al.) of mouse serum, washed in PBS, and then incubated with FITC-labeled goat anti-mouse IgG (whole molecule; Sigma-Aldrich, St. Louis, MO, USA). Anti-dsDNA autoantibodies were measured using serial dilutions and analyzed by *Crithidia lucillae* kinetoplast staining (The Binding Site, San Diego, CA, USA). Slides were viewed using fluorescent microscopy.

Statistical Analysis

Results are shown as the mean $\pm$ SEM. Differences between genders were determined by Student's *t* test and ANOVA analysis was performed for multiple comparisons. Results were considered statistically significant for *P*<0.05.

Results

Adiponectin-deficient mice in the MRL and MRL-*lpr* backgrounds were developed and maintained on normal mouse chow. MRL is a background strain of mouse that is widely accepted to be more susceptible to developing an autoimmune phenotype than the C57BL/6 strain [31]. The phenotype of the *lpr* mice results from a spontaneous mutation in the Fas antigen gene and displays both systemic autoimmunity and lymphoproliferative disease characterized mainly by lymphadenopathy and splenomegaly [32, 33]. Mice of each

genotype including MRL, MRL-*apn*^{-/-}, MRL-*lpr*, and MRL-*lpr.apn*^{-/-} were sacrificed and analyzed at 8 or 20 weeks of age. There was no difference between serum adiponectin levels in MRL and MRL-*lpr* mice. Serum adiponectin levels were not detectable in adiponectin-deficient MRL-*lpr.apn*^{-/-} mice and the signal in MRL-*apn*^{-/-} mice was not statistically significant. For each strain of mice with a functionally intact gene encoding adiponectin, female mice had higher circulating adiponectin levels compared to male mice at both age groups (Table I and data not shown). Circulating adiponectin levels were comparable to levels previously published in the C57BL/6 strain, and were higher than MRL or MRL-*lpr* mice.

Lymphadenopathy and splenomegaly are observed in mice with *lpr* mutations. MRL-*lpr* mice displayed lymph-adenopathy that was much more pronounced at 20 weeks of age compared to 8 weeks of age (Fig. 1a). The degree of lymphadenopathy was much more severe in the adiponectin-deficient MRL-*lpr* strain (MRL-*lpr.apn*^{-/-}) than in the MRL-*lpr* strain. With both the MRL-*lpr* and MRL-*lpr.apn*^{-/-} strains, the degree of lymphadenopathy was far greater in female than in male mice. Adiponectin deficiency had no impact on lymph node weight in wild type C57BL/6 or MRL mice.

A similar trend was observed in spleen size except that the splenomegaly phenotype was most readily apparent at the 20-week time point (Fig. 1b). Splenomegaly in MRL-*lpr* mice was relatively modest, but adiponectin deficiency in MRL-*lpr* mice resulted in a profound enlargement of the spleen at the 20-week time point. The exacerbated splenomegaly was particularly evident in female MRL-*lpr.apn*^{-/-} mice. In contrast, adiponectin deficiency alone did not affect spleen mass in mice on the C57BL/6 or MRL background strain.

Analysis of ANA titer was determined using serial dilutions of serum. Averages of titer indices are reported in Fig. 2. In female mice, ANA titers were markedly elevated at 8 weeks of age in adiponectin-deficient MRL-*lpr* mice compared to MRL-*lpr* mice (Fig. 2a). However, adiponectin deficiency had no effect on ANA titers in the MRL background. At the 8-week time point, male mice uniformly showed low ANA titers that did not significantly differ between strains in the MRL background (Fig. 2b). However, at 20 weeks of age, MRL-*lpr* mice that were deficient for adiponectin displayed elevated ANA titers relative to the parental MRL-*lpr* strain (Fig. 2b). Adiponectin deficiency had no effect on ANA titer in 20-week-old MRL mice. The SLE specific marker dsDNA was also quantified using serial dilutions of serum. As expected, female MRL-*lpr* mice had increased anti-dsDNA compared to males (Fig. 2c). In addition, female MRL-*lpr.apn*^{-/-} mice had significantly increased anti-dsDNA compared to female MRL-*lpr* mice (Fig. 2c).

Glomerulonephropathy is common in SLE, and is also a prominent characteristic of the MRL-*lpr* mouse [34]. Therefore, kidneys were examined for renal abnormalities. Glomeruli of 20-week-old female MRL-*lpr.apn*^{-/-} mice were significantly larger than their female MRL-*lpr* counterparts ($P < 0.005$; Fig. 3a). In contrast, adiponectin deficiency had no effect on the size of glomeruli in the MRL background. Male MRL-*lpr.apn*^{-/-} mice also showed significantly increased glomerular tuft size compared to male MRL-*lpr* mice at both timepoints ($P < 0.001$), but the magnitude of the enlargement was less than that observed in the corresponding female strains of mice (data not shown). Crescent formation was observed in about 35% of glomeruli in female MRL-*lpr.apn*^{-/-} mice (arrows in Fig. 3c) and quantification revealed a statistically significant increase compared to MRL-*lpr* mice which showed virtually no crescents (Fig. 3b). Immunofluorescent staining of frozen sections showed that 20-week-old female MRL-*lpr.apn*^{-/-} mice had significant IgG deposits within the kidney compared to the other genotypes analyzed (Fig. 3c). In addition, staining for complement C3 was also performed in 20-week-old female mice and revealed little to no staining in glomeruli of MRL or MRL-*apn*^{-/-} mice, and significant staining in MRL-*lpr*

mice which appeared to be exacerbated in the adiponectin-deficient MRL-*lpr* mice (Fig. 3c). Staining with PAS revealed mesangial matrix expansion and slight hypocellularity in enlarged glomeruli of 20 week old female MRL-*lpr.apn*^{-/-} mice compared to female MRL-*lpr* mice (Fig. 3c). Male mice exhibited a lesser degree of kidney abnormalities (data not shown). In addition to the crescents, presence of infiltrating inflammatory cells could be observed in kidneys of female MRL-*lpr.apn*^{-/-} mice stained with PAS, providing further evidence of disease progression in this model (Fig. 3c).

Discussion

Studies in experimental models have shown that adiponectin has an anti-inflammatory function. With regard to autoimmune phenotypes, adiponectin has been shown to promote apoptotic cell clearance by macrophages [17]. In addition, induction of adiponectin by peroxisome proliferator-activator receptor agonists has been shown to be beneficial for treatment of lupus and lupus-associated atherosclerosis in mouse models [35]. However, it has not been clear whether adiponectin deficiency is sufficient to induce an autoimmune phenotype or whether adiponectin deficiency exacerbates the inflammatory phenotype once autoimmunity is established. Since an autoimmune phenotype has not been reported for adiponectin deficiency in mice on a C57BL/6 background, we generated mice lacking adiponectin on the autoimmune-susceptible MRL background and compared the phenotype of this strain with MRL-*lpr* mice lacking adiponectin. Here, we report that adiponectin-deficient mice on the MRL background do not develop autoimmune phenotypes, but adiponectin deficiency in MRL-*lpr* mice displayed marked lupus-like disease parameters compared to MRL-*lpr* mice. Compared with the parental MRL-*lpr* strain, MRL-*lpr.apn*^{-/-} mice displayed increased lymphadenopathy, splenomegaly, ANA titer, and glomerular tuft size. Furthermore, these phenotypes were more pronounced in female mice versus male mice of the same genotype.

Adiponectin was initially termed ACRP for *Adipocyte Complement-Related Protein* based upon its homology to the complement protein C1q [36]. Similar to C1q, adiponectin is abundantly present in serum and it multimerizes leading to the formation of high molecular weight structures that are the predominant circulating forms [37]. Our recent work has indicated that, like C1q, adiponectin has a “collectin-like” function in that it is capable of opsonizing dead cells and facilitating their clearance by macrophages [17]. It has also been reported that adiponectin can bind C1q and activate the complement pathway [38]. However, adiponectin deficiency does not phenocopy the autoimmune phenotype of C1q deficiency in the MRL strain background. Whereas C1q deficiency on a C57BL/6 background does not produce autoimmune phenotype, C1q deficiency bred to the MRL background leads to an increase in the ANA titer and the development of glomerulonephritis [31]. Although we did not observe autoimmune phenotypes in adiponectin-deficient mice on the MRL background, adiponectin deficiency exacerbated the inflammatory phenotypes of MRL-*lpr* mice. Taken together, it appears that C1q has a more dominant role in suppressing the autoimmune phenotype than adiponectin.

Our findings also suggest that elevated adiponectin levels do not promote autoimmune disease progression as has been suggested by some studies [26]. Adiponectin levels are reported to be increased in SLE patients with renal dysfunction but not in SLE patients with normal renal function [39]. Urine adiponectin levels have been reported to be elevated SLE patients only during renal flare [39], and it has been suggested that high molecular weight adiponectin may contribute to this renal inflammation [40]. In contrast, a recent study has reported no significant difference in adiponectin levels of SLE patients versus healthy controls, whereas levels of the adipocyte-derived cytokine leptin are increased [41]. Finally, another study showed that adiponectin levels are increased in patients with SLE, but levels

are decreased in SLE patients displaying insulin resistance [27]. In contrast, the current findings and our previous studies [17, 35] show that the deficiency of adiponectin in a lupus model results in exacerbated disease. Therefore, the elevation of adiponectin in chronic autoimmune disease may reflect a compensatory change in the level of this cytokine to combat the inflammatory state. It is also possible that autoimmune disease states result in the development of “adiponectin-resistance” that leads to an elevation of adiponectin levels. Adiponectin resistance has been noted in human tissue and animal models [42–44], but the mechanisms underlying this resistant state have not been defined. There are many factors that impact the production levels of adiponectin including metabolic status, gender, age, and inflammatory state [26]. Thus, the differential impact of these factors on adiponectin expression may influence the severity of autoimmune disease.

Gender influenced the impact of adiponectin-deficiency on the phenotype of MRL-*lpr* mice. Glomerular size of female MRL-*lpr.apn*^{-/-} mice was greater compared to male MRL-*lpr.apn*^{-/-} mice. In addition, female MRL-*lpr.apn*^{-/-} mice displayed significantly greater lymphadenopathy and splenomegaly compared to males with the same genotype. It is well established that a significantly larger fraction of lupus patients are female [45]. Thus, it is conceivable that the role of adiponectin in suppressing autoimmunity is of greater importance in females than males. Although the impact of gender on adiponectin levels in SLE patients has not been studied, adiponectin levels are higher in females compared with males in the general population [8].

In conclusion, we have demonstrated that adiponectin deficiency in mice is not sufficient to initiate an autoimmune phenotype even in the “permissive” MRL background. However, adiponectin deficiency in the context of a mouse model of established autoimmunity leads to more robust disease when compared to adiponectin-sufficient controls. The effects of adiponectin on the modulation of autoimmune phenotype occurred in a gender specific manner; adiponectin deficiency had a more profound effect in promoting an autoimmune phenotype in female mice.

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References

1. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res.* 2005; 96:939–49. [PubMed: 15890981]
2. Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Walsh K. Obesity, adiponectin and vascular inflammatory disease. *Curr Opin Lipidol.* 2003; 14:561–6. [PubMed: 14624132]
3. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun.* 1999; 257:79–83. [PubMed: 10092513]
4. Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, et al. Adiponectin as a biomarker of the metabolic syndrome. *Circ J.* 2004; 68:975–81. [PubMed: 15502375]
5. Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J, et al. Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. *Biochem Biophys Res Commun.* 2003; 301:1045–50. [PubMed: 12589818]
6. Kappes A, Loffler G. Influences of ionomycin, dibutyl-cycloAMP and tumour necrosis factor- α on intracellular amount and secretion of apM1 in differentiating primary human preadipocytes. *Horm Metab Res.* 2000; 32:548–54. [PubMed: 11246823]

7. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes*. 2001; 50:2094–9. [PubMed: 11522676]
8. Isobe T, Saitoh S, Takagi S, Takeuchi H, Chiba Y, Katoh N, et al. Influence of gender, age and renal function on plasma adiponectin level: the Tanno and Sobetsu study. *Eur J Endocrinol*. 2005; 153:91–8. [PubMed: 15994750]
9. Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, et al. Adiponectin and protection against type 2 diabetes mellitus. *Lancet*. 2003; 361:226–8. [PubMed: 12547549]
10. Iwashima Y, Horio T, Kumada M, Suzuki Y, Kihara S, Rakugi H, et al. Adiponectin and renal function, and implication as a risk of cardiovascular disease. *Am J Cardiol*. 2006; 98:1603–8. [PubMed: 17145218]
11. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA*. 2004; 291:1730–7. [PubMed: 15082700]
12. Atzmon G, Pollin TI, Crandall J, Tanner K, Schechter CB, Scherer PE, et al. Adiponectin levels and genotype: a potential regulator of life span in humans. *J Gerontol A Biol Sci Med Sci*. 2008; 63:447–53. [PubMed: 18511746]
13. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med*. 2002; 8:731–7. [PubMed: 12068289]
14. Ohashi K, Kihara S, Ouchi N, Kumada M, Fujita K, Hiuge A, et al. Adiponectin replenishment ameliorates obesity-related hypertension. *Hypertension*. 2006; 47:1108–16. [PubMed: 16651465]
15. Okamoto Y, Folco E, Minami M, Wara A, Feinberg M, Sukhova G, et al. Adiponectin inhibits the production of CXC receptor 3 chemokine ligands in macrophages and reduces T-lymphocyte recruitment in atherosclerosis. *Circ Res*. 2008; 102:218–25. [PubMed: 17991878]
16. Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, et al. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation*. 2002; 106:2767–70. [PubMed: 12451000]
17. Takemura Y, Ouchi N, Shibata R, Aprahamian T, Kirber MT, Summer RS, et al. Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. *J Clin Invest*. 2007; 117:375–86. [PubMed: 17256056]
18. Shibata R, Ouchi N, Ito M, Kihara S, Shiojima I, Pimentel DR, et al. Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat Med*. 2004; 10:1384–9. [PubMed: 15558058]
19. Shibata R, Ouchi N, Kihara S, Sato K, Funahashi T, Walsh K. Adiponectin stimulates angiogenesis in response to tissue ischemia through stimulation of amp-activated protein kinase signaling. *J Biol Chem*. 2004; 279:28670–4. [PubMed: 15123726]
20. Shibata R, Sato K, Pimentel DR, Takemura Y, Kihara S, Ohashi K, et al. Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nat Med*. 2005; 11:1096–103. [PubMed: 16155579]
21. Engeli S, Feldpausch M, Gorzelniak K, Hartwig F, Heintze U, Janke J, et al. Association between adiponectin and mediators of inflammation in obese women. *Diabetes*. 2003; 52:942–7. [PubMed: 12663465]
22. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA*. 2003; 289:1799–804. [PubMed: 12684358]
23. Krakoff J, Funahashi T, Stehouwer CD, Schalkwijk CG, Tanaka S, Matsuzawa Y, et al. Inflammatory markers, adiponectin, and risk of type 2 diabetes in the Pima Indian. *Diab Care*. 2003; 26:1745–51.
24. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation*. 2003; 107:671–4. [PubMed: 12578865]
25. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, et al. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood*. 2000; 96:1723–32. [PubMed: 10961870]

26. Fantuzzi G. Adiponectin and inflammation: consensus and controversy. *J Allergy Clin Immunol.* 2008; 121:326–30. [PubMed: 18061654]
27. Sada KE, Yamasaki Y, Maruyama M, Sugiyama H, Yamamura M, Maeshima Y, et al. Altered levels of adipocytokines in association with insulin resistance in patients with systemic lupus erythematosus. *J Rheumatol.* 2006; 33:1545–52. [PubMed: 16881111]
28. Schaffler A, Ehling A, Neumann E, Herfarth H, Tarner I, Scholmerich J, et al. Adipocytokines in synovial fluid. *JAMA.* 2003; 290:1709–10. [PubMed: 14519703]
29. Furuta M, Tamai M, Hanabusa T, Yamamoto Y, Nanjo K, Sanke T. Serum adiponectin is associated with fasting serum C-peptide in non-obese diabetic patients. *Diabetes Res Clin Pract.* 2006; 72:302–7. [PubMed: 16442181]
30. Perseghin G, Lattuada G, Danna M, Sereni LP, Maffi P, De Cobelli F, et al. Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. *Am J Physiol Endocrinol Metab.* 2003; 285:E1174–81. [PubMed: 12933352]
31. Bickerstaff MC, Botto M, Hutchinson WL, Herbert J, Tennent GA, Bybee A, et al. Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. *Nat Med.* 1999; 5:694–7. [PubMed: 10371509]
32. Cohen PL, Eisenberg RA. Lpr and gld: single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu Rev Immunol.* 1991; 9:243–69. [PubMed: 1910678]
33. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature.* 1992; 356:314–7. [PubMed: 1372394]
34. Theofilopoulos AN, Dixon FJ. Murine models of systemic lupus erythematosus. *Adv Immunol.* 1985; 37:269–390. [PubMed: 3890479]
35. Aprahamian T, Bonegio RG, Richez C, Yasuda K, Chiang LK, Sato K, et al. The peroxisome proliferator-activated receptor gamma agonist rosiglitazone ameliorates murine lupus by induction of adiponectin. *J Immunol.* 2009; 182:340–6. [PubMed: 19109165]
36. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem.* 1995; 270:26746–9. [PubMed: 7592907]
37. Suzuki S, Wilson-Kubalek EM, Wert D, Tsao TS, Lee DH. The oligomeric structure of high molecular weight adiponectin. *FEBS Lett.* 2007; 581:809–14. [PubMed: 17292892]
38. Peake PW, Shen Y, Walther A, Charlesworth JA. Adiponectin binds C1q and activates the classical pathway of complement. *Biochem Biophys Res Commun.* 2008; 367:560–5. [PubMed: 18179772]
39. Rovin BH, Song H, Hebert LA, Nadasdy T, Nadasdy G, Birmingham DJ, et al. Plasma, urine, and renal expression of adiponectin in human systemic lupus erythematosus. *Kidney Int.* 2005; 68:1825–33. [PubMed: 16164660]
40. Song H, Chan J, Rovin BH. Induction of chemokine expression by adiponectin in vitro is isoform dependent. *Transl Res.* 2009; 154:18–26. [PubMed: 19524870]
41. Vadacca M, Margiotta D, Rigon A, Cacciapaglia F, Coppolino G, Amoroso A, et al. Adipokines and systemic lupus erythematosus: relationship with metabolic syndrome and cardiovascular disease risk factors. *J Rheumatol.* 2009; 36:295–7. [PubMed: 19132788]
42. Bruce CR, Mertz VA, Heigenhauser GJ, Dyck DJ. The stimulatory effect of globular adiponectin on insulin-stimulated glucose uptake and fatty acid oxidation is impaired in skeletal muscle from obese subjects. *Diabetes.* 2005; 54:3154–60. [PubMed: 16249439]
43. Panchapakesan U, Chen XM, Pollock CA. Drug insight: thiazolidinediones and diabetic nephropathy—relevance to renoprotection. *Nat Clin Pract Nephrol.* 2005; 1:33–43. [PubMed: 16932362]
44. Mullen KL, Smith AC, Junkin KA, Dyck DJ. Globular adiponectin resistance develops independently of impaired insulin-stimulated glucose transport in soleus muscle from high-fat fed rats. *Am J Physiol Endocrinol Metab.* 2007; 293:E83–90. [PubMed: 17356008]
45. Zandman-Goddard G, Peeva E, Shoenfeld Y. Gender and autoimmunity. *Autoimmun Rev.* 2007; 6:366–72. [PubMed: 17537382]

Fig. 1.

Adiponectin deficiency results in increased lymphoproliferative phenotype in female MRL-*lpr* mice. **a** All superficial cervical lymph nodes were collected at 8 or 20 weeks of age from each strain of mice and weighed wet. Female MRL-*lpr.apn*^{-/-} mice have significantly larger lymph nodes size compared to female MRL-*lpr* mice, as well as male MRL-*lpr.apn*^{-/-} mice (***P*<0.01 (*n*=5–8)). Male MRL-*lpr.apn*^{-/-} mice have increased lymph node weight compared to male MRL-*lpr* mice **P*<0.05. **b** Whole spleen was excised from each strain at 8 and 20 weeks of age. Spleen weights from female MRL-*lpr.apn*^{-/-} mice are increased compared to female MRL-*lpr* mice, as well as male MRL-*lpr.apn*^{-/-} mice (***P*<0.01), and male MRL-*lpr.apn*^{-/-} mice have significantly increased spleen size compared to male MRL-*lpr* mice **P*<0.05

Fig. 2. Autoantibody titer is elevated in lupus mice deficient for adiponectin. Circulating levels of anti-nuclear antibodies (ANA) were determined by analyzing serial dilutions of serum on slides coated with Hep-2 cells. **a** A significant increase in ANA titer was observed in 8 week old female MRL-*lpr*. *apn*^{-/-} mice (*n*=10) compared to female MRL-*lpr* mice (*n*=7). **b** Similar effects were observed in 20-week-old male MRL-*lpr*. *apn*^{-/-} mice (*n*=8) compared to age-matched male MRL-*lpr* mice (*n*=8). **c** Circulating levels of anti-dsDNA antibodies were examined by analysis of serial serum dilutions on *C. lucillae*. Anti-dsDNA was significantly increased in 20-week-old female MRL-*lpr*.*apn*^{-/-} mice (*n*=10) compared to female MRL-*lpr* mice (*n*=7) (**P*<0.01)

Fig. 3.

Exacerbated renal abnormalities in MRL-*lpr* mice lacking adiponectin. **a** Glomerular tuft size was quantified and is significantly increased in 20-week-old female MRL-*lpr.apn*^{-/-} mice compared to MRL-*lpr* mice. **b** Crescent formation was quantified to be significantly increased in 20-week-old female MRL-*lpr.apn*^{-/-} mice compared to control groups. **c** Immunofluorescent microscopy of glomeruli from 20-week-old female mice stained for immune complex (*IgG*) deposition, complement (*C3*). Representative images show that adiponectin deficiency on an MRL background has similar staining as MRL mice. However, adiponectin deficiency in combination with a lupus phenotype reveals robust staining for both *IgG*, and *C3* within the glomeruli of MRL-*lpr.apn*^{-/-} mice compared to MRL-*lpr* mice ($n=5-8$). Periodic Acid Schiff (*PAS*) staining shows that MRL-*lpr.apn*^{-/-} mice have exacerbated kidney disease compared to MRL-*lpr* mice. Glomerular crescents are present in MRL-*lpr.apn*^{-/-} mice (arrows) (* $P<0.05$, ** $P<0.01$)

Table 1Serum adiponectin levels ($\mu\text{g/ml}$)

	C57BL/6	MRL	MRL- <i>apn</i>	MRL- <i>lpr</i>	MRL- <i>lpr.apn</i>
Female	22.6 $\pm$ 5.67	15.7 $\pm$ 1.79	1.41 $\pm$ 1.65	14.4 $\pm$ 2.52	nd
Male	15.2 $\pm$ 1.67	8.73 $\pm$ 1.24	2.94 $\pm$ 2.75	9.05 $\pm$ 0.73	nd