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The Peroxisome Proliferator-Activated Receptor $\gamma$ Agonist Rosiglitazone Ameliorates Murine Lupus by Induction of Adiponectin $^1$

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Systemic lupus erythematous (SLE) is an inflammatory autoimmune disease for which current therapy is suboptimal. SLE is characterized by autoantibody production, with renal disease and premature atherosclerosis being common and severe manifestations causing appreciable morbidity and mortality. Peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) agonists are widely used in the treatment of diabetes mellitus for their insulin-sensitizing properties, but also have immunomodulatory effects. In this report, we show that the PPAR$\gamma$ agonist rosiglitazone reduces autoantibody production, renal disease, and atherosclerosis in murine models of SLE. The beneficial effect of rosiglitazone on SLE manifestations depends on the induction of adiponectin, because rosiglitazone has no effect on autoantibody production or renal disease in lupus mice that lack adiponectin. In addition, lupus mice that lack adiponectin develop more severe disease than adiponectin-sufficient lupus mice, indicating that endogenous adiponectin is involved in regulating disease activity. Furthermore, administration of exogenous adiponectin ameliorates disease.

These experiments suggest that PPAR$\gamma$ agonists may be useful agents for the treatment of SLE. They also demonstrate that induction of adiponectin is a major mechanism underlying the immunomodulatory effects of PPAR$\gamma$ agonists. The Journal of Immunology, 2009, 182: 340–346.

The etiology of lupus is complex, with genetic predisposition, gender, environment, and stochastic factors all contributing (1). End-organ disease, in particular nephritis, is due to the dysfunction of multiple effector pathways including immune complex deposition and complement activation, T cell, granulocyte, and macrophage activation, and various proinflammatory cytokines (2). Premature atherosclerosis associated with systemic lupus erythematosus (SLE) $^3$ leads to early onset coronary artery disease, stroke, and peripheral vascular disease. This vascular disease is a major cause of morbidity and mortality in SLE patients, with poorly understood lupus-specific risk factors contributing substantially to its pathogenesis (3). Current treatment of SLE comprises nonspecific immunosuppression with frequent serious side-effects, and clinical improvement in response to this treatment is often incomplete (4). Thus, new and less toxic therapies are required.

Thiazolidinediones are high affinity ligands and agonists for peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) (5), and are used clinically for the treatment of type 2 diabetes mellitus because of their insulin-sensitizing properties. The two thiazolidinediones currently available in the U.S. for clinical use are rosiglitazone and pioglitazone (6). PPAR$\gamma$, a member of the nuclear receptor superfamily, plays important roles in adipocyte differentiation and in lipid and carbohydrate metabolism (7, 8). In addition, PPAR$\gamma$ is involved in the regulation of inflammation and immunity. In vitro, PPAR$\gamma$ agonists induce a variety of anti-inflammatory and immunomodulatory effects in diverse cell types including macrophages, dendritic cells, T cells and endothelial cells (9–11). In vivo, PPAR$\gamma$ agonists are effective in reducing disease manifestations in rodent models of human inflammatory and autoimmune diseases including inflammatory bowel disease, multiple sclerosis, and arthritis (10, 11), and a recent human clinical study reported that rosiglitazone is beneficial in the treatment of mild to moderate ulcerative colitis (12). However, the immunomodulatory mechanisms of PPAR$\gamma$ action are incompletely understood in vivo.

The effectiveness of PPAR$\gamma$ agonists in models of lupus has not been examined previously. Because the in vitro immunomodulatory effects of PPAR$\gamma$ impact pathways that may be involved in the pathogenesis of lupus, we hypothesized that PPAR$\gamma$ agonists would be beneficial in the treatment of SLE. In this report, we used two mouse models of lupus to determine the efficacy of rosiglitazone treatment on SLE.

Materials and Methods

Mice

Gld:apoE$^{-/-}$ mice and adiponectin-deficient (apo$^{-/-}$) mice, both on a C57BL/6 genetic background, have been described elsewhere (13, 14). To obtain the experimental MRL-lpr/lpr,apo$^{-/-}$ (MRL-lpr) and MRL-lpr/lpr,apo$^{-/-}$ (MRL-lpr,apo$^{-/-}$) mice, we first generated MRL-apa$^{-/-}$ mice by backcrossing C57BL/6 apa$^{-/-}$ mice to the MRL/MpJ strain (The Jackson Laboratories) for six generations. We then intercrossed the MRL-apa$^{-/-}$ mice with MRL-lpr mice (The Jackson Laboratories) to obtain the experimental MRL-lpr and MRL-lpr,apa$^{-/-}$ mice. Studies were reviewed and approved by the Institutional Animal Care and Use Committee at Boston University School of Medicine.
Rosiglitazone administration

Seven-wk-old male and female gldapoE<sup>−/−</sup> mice and 6-wk-old female MRL-lpr and MRL-lpr<sup>apn</sup> mice received either normal diet or normal diet supplemented with rosiglitazone at a dose of 10 mg/kg/day (GlaxoSmithKline; diet prepared by Harlan-Teklad Special Diets). Food intake and body weight were monitored weekly. We killed the mice after 12 wk on the diets and at that time obtained blood by cardiac puncture, excised and weighed the spleen and submandibular lymph nodes, excised the kidneys, and isolated the aortae.

Adenoviral vector administration

Four × 10<sup>8</sup> plaque-forming units of an adenovirus expressing mouse adiponectin or an adenovirus expressing β-galactosidase (15) were administered by tail vein injection to 12 wk old male and female gldapoE<sup>−/−</sup> mice. As adenovirus experiments are necessarily short-term, these mice were given a high-fat Western diet for 5 wk before infection to induce a more rapidly progressive disease phenotype as described elsewhere (13). The mice were killed 14 days after infection and tissues were collected for analysis.

Analysis of atherosclerosis

Aortic atherosclerosis was analyzed as previously described (13). In brief, the vasculature was perfused and the aorta was isolated from the aortic arch to the iliac bifurcation. It was opened longitudinally and fixed in 10% neutral buffered formalin for 24 h. After fixation, the aortae were stained with Oil Red O solution to identify the atherosclerotic lesions. The aortae were photographed using an Olympus digital camera and atherosclerotic lesions were measured using Adobe Photoshop. The data are expressed as the amount of atherosclerosis relative to total aortic area.

Kidney histology

Kidney tissue was fixed with 10% formalin, embedded in paraffin, and sections were stained either with H&E or with Sirius red. Stained sections were coded and digitally photographed and analyzed by an investigator who was blinded to section identity. Glomerular cross-sectional area was determined using computer-assisted image analysis (Photoshop CS3) of randomly photographed low-power images of the renal cortex. The area of at least 25 glomeruli in each animal was measured to determine a mean glomerular area for that animal.

Glomerular cell count was determined using the same photographs used for the determination of area. Sections were stained with H&E and the blue-stained nuclei in the glomerular tuft were counted.

Crescents were identified by their typical histologic appearance on H&E-stained sections and the data are shown as the percentage of glomeruli with crescents. A total of 100 glomeruli per animal were examined for crescents.

Sirius red staining was performed to determine the extent of interstitial fibrosis within the kidney. The stained area was quantified using Photoshop CS3 and the data expressed as a fibrosis index which is the percentage of the cortical area that stains with Sirius red.

Serological assays

Serum antinuclear Ab (ANA) titer was measured by immunofluorescence using Hep-2-coated-slides (The Binding Site) as described elsewhere (13). ANA titer is expressed as the ANA titer index as adapted from Komori et al. (16).
In brief, slides were incubated for 1 h with serial log-scale serum dilutions and immunofluorescence intensity was scored as follows: 0, not stained with \(1 \times 10^2\) dilution; 1, stained with \(1 \times 10^2\) dilution; 2, stained with \(1 \times 10^3\) dilution; 3, faintly stained with \(1 \times 10^3\) dilution; 4, stained with \(1 \times 10^4\) dilution; 5, faintly stained with \(1 \times 10^4\) dilution; 6, stained with \(3 \times 10^4\) dilution; 7, faintly stained with \(3 \times 10^4\) dilution; and 8, stained with \(9 \times 10^4\) dilution. Serum adiponectin concentrations were measured by ELISA according to the manufacturer’s protocol (B-Bridge International).

Flow cytometry

Splenocytes from MRL-lpr.apn\(^{-/-}\) mice were labeled with anti-CD4-FITC, anti-CD8-PE, and anti-CD19-FITC in combination with biotinylated anti-CD69 and streptavidin-PE-Cy5 (all from BD Biosciences). Immunofluorescence was measured using a FACSscan flow cytometer (BD Biosciences) and the data analyzed with FlowJo software (Tree Star).
Statistical analysis

Data are reported as the mean ± SEM. Differences between groups were determined by two-tailed, unpaired Student’s t tests, except in the case of the analysis of ANA titer where the Mann-Whitney U test was used. p values <0.05 were considered statistically significant.

Results

Rosiglitazone reduces disease manifestations in gld.apoE−/− mice

We previously described a mouse model of premature atherosclerosis and SLE that was developed by breeding lupus-prone Fas ligand-deficient (gld) mice with atherosclerosis-prone apolipoprotein E-deficient (apoE−/−) mice, to create mice deficient in both Fas ligand and apolipoprotein E (gld. apoE−/−) (13). These mice develop a proliferative glomerulonephritis, ANA, and have more severe atherosclerosis than apoE−/− mice. To test the hypothesis that PPARγ agonists would be effective in the treatment of SLE, gld. apoE−/− mice were maintained on normal diet or normal diet supplemented with the PPARγ agonist rosiglitazone for 12 wk, starting at 6 wk of age and then disease parameters were measured. A. Rosiglitazone treatment decreases ANA titer in MRL-lpr mice, but not in MRL-lpr.apn−/− mice. Rosiglitazone treatment decreases glomerular size (B) and glomerular cell number (C) in MRL-lpr mice, but not in MRL-lpr.apn−/− mice. Adiponectin deficiency exacerbates renal disease in MRL-lpr mice as shown by D, an increase in glomerular crescents and E, an increase in fibrosis index. F. Representative photographs showing a decrease in glomerular size in rosiglitazone-treated MRL-lpr mice but not in rosiglitazone-treated MRL-lpr.apn−/− mice, and an increase in glomerular size and cellularity in adiponectin-deficient mice compared with adiponectin-sufficient mice. Arrows indicate glomerular crescents. G. Representative photographs of kidney sections stained with Sirius red which stains areas of fibrosis red. Increased fibrosis is seen in adiponectin-deficient mice compared with adiponectin-sufficient mice. *, p < 0.05; **, p < 0.01; ***, p < 0.001.
in a reduction in both of these parameters (Fig. 1, E–G). Aortic atherosclerosis was also substantially reduced in the gld.apoE/apoE−/− mice receiving rosiglitazone compared with the gld.apoE/apoE−/− mice receiving normal diet (Fig. 1, H and I). Overall, these results indicate that rosiglitazone has beneficial effects on disease development in a mouse model of lupus that is associated with accelerated atherosclerosis.

Adiponectin reduces disease manifestations in gld.apoE−/− mice

Previously, we reported that short-term administration of adiponectin reduced disease activity in the MRL-lpr lupus model (18). Because PPARγ agonists are known to up-regulate adiponectin (19), we hypothesized that the effects of rosiglitazone in the gld.apoE−/− model might be mediated by the induction of this anti-inflammatory adipokine (20). We first measured adiponectin levels in the sera of the gld.apoE−/− experimental mice shown in Fig. 1, and found that mice receiving rosiglitazone for 12 wk had significantly higher levels of serum adiponectin than mice not receiving rosiglitazone (Fig. 2A). To determine whether adiponectin could mediate protective effects in the gld.apoE−/− model, we administered adiponectin by adenoviral vector (adeno-adiponectin) which increases the serum levels of all three of the major oligomeric forms of adiponectin normally found in serum (15). We found that a 2-wk treatment with adeno-adiponectin significantly reduced serum ANA levels (Fig. 2B), glomerular size (Fig. 2C), and glomerular cell count (Fig. 2D) compared with treatment with a control vector (adeno-βgalactosidase). Thus, short-term administration of adiponectin is sufficient to reduce disease manifestations in the gld.apoE−/− model.

Rosiglitazone reduces disease manifestations in the MRL-lpr lupus model by induction of adiponectin

To provide causal evidence that adiponectin up-regulation is an important mechanism underlying the beneficial effects of rosiglitazone, we compared the efficacy of rosiglitazone in MRL-lpr mice with adeno-adiponectin (21), with the effects in MRL-lpr mice deficient in adiponectin (MRL-lpr.apn−/−). Treatment started at 6 wk of age and continued for a further 12 wk, at which time the analyses were performed. Body weight was not different between the rosiglitazone-treated and untreated mice in either the MRL-lpr cohorts or the MRL-lpr.apn−/− cohorts demonstrating that the presence of rosiglitazone in the diet did not result in decreased food intake (Fig. 3A). To confirm that the mice had indeed ingested the rosiglitazone diet and that the ingested rosiglitazone was active, we measured serum adiponectin levels in the MRL-lpr cohort. Serum adiponectin levels were approximately twice as high in the MRL-lpr mice that received rosiglitazone as compared with those that received only normal diet (Fig. 3B). This approach could not be used in the MRL-lpr.apn−/− cohort which lacks adiponectin so we instead measured the expression of CD69 on splenic CD4+ T, CD8+ T, and B cells. CD69 is an early activation molecule expressed on bone-marrow derived cells and is down-regulated by PPARγ agonists (22, 23). CD69 expression on splenic CD4+ T cells, CD8+ T cells, and B cells was significantly lower in the MRL-lpr.apn−/− mice that received rosiglitazone compared with those that received only normal diet (Fig. 3, C and D), indicating that rosiglitazone was functional in these mice.

Next we evaluated the autoimmune manifestations in the two groups. ANA titer was reduced and lymph node size was smaller in MRL-lpr mice treated with rosiglitazone compared with untreated MRL-lpr mice, whereas no significant effect of rosiglitazone on these parameters was seen in MRL-lpr.apn−/− mice (Fig. 4A and data not shown). Renal disease was ameliorated in the MRL-lpr mice treated with rosiglitazone compared with untreated MRL-lpr mice, as shown by a reduction in glomerular area and glomerular cell count (Fig. 4, B and C). In contrast, these parameters were not affected by rosiglitazone treatment in MRL-lpr.apn−/− mice (Fig. 4, B and C). Strikingly, the renal disease was much more severe in the MRL-lpr.apn−/− mice than in the MRL-lpr adiponectin-sufficient mice (Fig. 4, B and C). To evaluate the renal disease in more detail, we also measured the percentage of glomerular crescents and extent of renal fibrosis, both of which are indicators of more severe renal injury (17). Whereas the adiponectin-sufficient mice had little evidence of either crescents or fibrosis, the adiponectin-deficient mice had appreciable amounts of both (Fig. 4, D–G). Overall, the data show that rosiglitazone ameliorates disease in MRL-lpr mice and that this effect is dependent on adiponectin. The data further demonstrate that endogenous adiponectin plays an important role in controlling the severity of disease.

Discussion

In this study, we show that the PPARγ agonist rosiglitazone ameliorates the autoimmune phenotype in two experimental models of SLE. Rosiglitazone significantly increased serum adiponectin levels; it decreased ANA titer, lupus-nephritis, and lupus-associated atherosclerosis after 12 wk of treatment. The importance of adiponectin was determined by observations that short-term overexpression of adiponectin had beneficial effects on the lupus phenotype. We further demonstrate that adiponectin is necessary for these beneficial effects by showing that rosiglitazone treatment has no effect on an adiponectin-deficient mouse model of lupus. Taken together, these results demonstrate that induction of adiponectin is a major in vivo mechanism whereby this beneficial effect of rosiglitazone in a SLE model is mediated.

Adiponectin is an abundant circulating adipocyte-derived cytokine with multiple biological activities including enhancing insulin sensitivity and protecting against atherosclerosis and other cardiovascular pathologies (14, 15, 24–28). These effects are thought to be mediated by adiponectin binding to specific cell surface receptors, and activation of signaling pathways within the target cell (29, 30). Adiponectin has a variety of anti-inflammatory properties (20, 31). Clinical studies have demonstrated a correlation between low plasma adiponectin concentrations and high levels of C-reactive protein, an established inflammatory marker in various populations (32–35). Experimental studies show that adiponectin reduces TNF-α production in response to various stresses in plasma, adipose tissue, vascular wall, heart, and liver (14, 26, 36, 37). In vitro, adiponectin inhibits the production of proinflammatory cytokines, and enhances the production of anti-inflammatory cytokines, by macrophages and dendritic cells in response to LPS (38, 39). Moreover, adiponectin decreases NF-κB activity in a macrophage cell line in response to TLR2, TLR4, and TLR9 ligands (40).

Despite the overall anti-inflammatory properties of adiponectin, the regulation of the production of adiponectin and other adipocytokines in inflammatory conditions is complex and incompletely understood. Obesity is associated with chronic low-grade inflammation of adipose tissue as well as systemic inflammation and low serum levels of adiponectin are found in obese individuals (41). This has led to the concept that proinflammatory factors present in obesity suppress adiponectin production and the low levels of adiponectin lead both to insulin resistance and to the exacerbation of inflammation (42). However, in a number of other inflammatory conditions including SLE, rheumatoid arthritis, and inflammatory bowel disease, elevated adiponectin levels are found, with some reports showing a positive correlation between adiponectin levels and markers of inflammation (43–46). Thus, the nature of the inflammatory response differs between obesity and these other conditions.
conditions, at least in terms of adiponectin regulation. This apparent paradox has recently been reviewed and it is evident that more work needs to be done to experimentally address the various possible explanations for these findings (42). It is not clear whether the increased adiponectin levels seen in these autoinflammatory conditions represents a regulatory mechanism to dampen down the inflammatory response or whether in some situations adiponectin might have proinflammatory activity. Our data suggest that in the case of SLE the anti-inflammatory effects of adiponectin are likely to be most relevant. In patients with SLE, obesity may contribute to the overall extent of inflammation. It has been reported that obesity in patients with lupus is independently associated with markers of inflammation and also with impaired functional capacity (47). The metabolic syndrome is not uncommon in patients with SLE (48) and insulin resistance, a component of the metabolic syndrome, is higher in lupus patients than in controls (43, 49). There is limited data regarding other adipocytokines in SLE. However, serum levels of leptin, a proinflammatory adipocytokine (20), are elevated in some lupus cohorts (43, 50) although not in all (51). The adipocytokine resistin has been proposed as a possible marker of inflammation in SLE, although resistin levels overall did not differ between SLE patients and controls (52).

There is considerable data linking impaired clearance of apoptotic debris to the pathogenesis of lupus and lupus nephritis, as well as to other systemic autoimmune and inflammatory conditions such as atherosclerosis (53). Thus, strategies to enhance safe clearance of this apoptotic material might prevent disease or ameliorate disease severity. Many of the more common autoantigens in SLE are components of either DNA/protein or RNA/protein multimolecular particles that are normally found in the cell nucleus, but which cluster and concentrate in “apoptotic blebs” at the surface of cells undergoing apoptosis (54, 55). These autoantigens, internalized into B cells or dendritic cells in the form of immune complexes or microparticles, may act as a trigger for cell death and contribute to the development of autoimmunity (56). They have been shown that adiponectin is able to bind to early apoptotic bodies and mediate their clearance by macrophages (18), suggesting the possibility that adiponectin might be able to bind to and sequester this immunostimulatory DNA- and RNA-containing material released from apoptotic cells. This possibility is supported by the fact that Clq, which is structurally very similar to adiponectin, is able to directly bind to DNA (57).

Our study suggests that treatment with the currently available PPARγ agonists may represent a novel therapeutic approach to SLE. Although this possibility is appealing, there are concerns about side-effects of these agents including fluid retention leading to edema, heart failure in some individuals and an increased risk of bone fractures (58, 59). One implication of our study is that it may be possible to develop new PPARγ agonists that specifically target PPARγ2 which, unlike the ubiquitously expressed PPARγ1 isoform, is expressed only in adipose tissue (6, 60, 61). Thus, by targeting PPARγ2 it may be possible to induce adiponectin expression by adipocytes while minimizing effects on other tissues. In summary, we show for the first time that a PPARγ agonist is able to reduce disease manifestations in mouse models of SLE, and that induction of adiponectin is a major in vivo mechanism whereby this beneficial effect is mediated. It will be important in future studies to determine whether this mechanism is also responsible for the observed beneficial effects of PPARγ agonists in other autoimmune diseases. Overall, these data suggest that PPARγ agonists, or other strategies to enhance adiponectin expression, may be useful agents for the treatment of SLE.


