Roles of Interleukin 17 in Angiotensin II Type 1 Receptor–Mediated Insulin Resistance

Kousei Ohshima, Masaki Mogi, Fei Jing, Jun Iwanami, Kana Tsukuda, Li-Juan Min, Jitsuo Higaki, Masatsugu Horiuchi

Abstract—Interleukin 17 (IL-17) is known to contribute to the pathogenesis of hypertension, atherosclerosis, and adipocyte differentiation; however, the roles of IL-17 in glucose metabolism remain to be elucidated. Angiotensin II type 1 receptor blockers improve insulin resistance at least in part because of the amelioration of inflammation. Therefore, we examined the possible roles of IL-17 in the pathogenesis of insulin resistance in type 2 diabetes mellitus using a mouse model, KK-Ay, and angiotensin II type 1 receptor–mediated insulin resistance. KK-Ay mice were administered control-IgG or anti-IL-17 antibody 5 times at a dose of 100 μg every second day by IP injection. KK-Ay mice were administered telmisartan for 2 weeks. C57BL/6J mice treated with angiotensin II infusion for 2 weeks were administered telmisartan or hydralazine. Insulin resistance was evaluated by oral glucose tolerance test, insulin tolerance test, and uptake of 2-[3H]deoxy-D-glucose in peripheral tissues. Serum IL-17 concentration in KK-Ay mice was significantly higher than that in C57BL/6J mice. Treatment of KK-Ay mice with anti–IL-17 antibody significantly increased 2-[3H]deoxy-D-glucose uptake in skeletal muscle but not in white adipose tissue and attenuated the increase in blood glucose level after a glucose load. Blockade of IL-17 enhanced the expression of adipocyte differentiation markers and adiponectin. Treatment with telmisartan decreased serum IL-17 concentration in KK-Ay and ameliorated angiotensin II–induced insulin resistance with a decrease in serum IL-17 level in C57BL/6J. In conclusion, IL-17 could play an important role in the pathogenesis of angiotensin II type 1 receptor–induced insulin resistance. (Hypertension. 2012; 59[part 2]:493-499.) • Online Data Supplement

Key Words: low-grade inflammation • interleukin 17 • insulin resistance • angiotensin II type 1 receptor blocker

There is increasing evidence that persistent low-grade inflammation could result in insulin resistance in target tissues, which plays a major role in the pathogenesis and progression of type 2 diabetes mellitus (T2DM) and subsequent cardiovascular disease. Studies in various diabetic and insulin-resistant states in humans demonstrated a link between chronic activation of proinflammatory signaling pathways and decreased insulin sensitivity. In addition, elevated levels of inflammatory markers, including interleukin (IL) 1β, IL-6, and C-reactive protein, have been reported to be predictive for the development of T2DM. Angiotensin II plays an important role in the pathogenesis of insulin resistance at least in part because of enhanced inflammation and oxidative stress via angiotensin II type 1 (AT1) receptor stimulation. We demonstrated previously that an AT1 receptor blocker (ARB) ameliorated insulin resistance in a T2DM mouse model, KK-Ay; however, the association between low-grade inflammation and the efficacy of ARB to attenuate insulin resistance is still unclear.

Recently, a proinflammatory cytokine, IL-17, produced by T-helper 17 (Th17) cells has been reported to be involved in the pathogenesis of atherosclerotic diseases and adipocyte differentiation and glucose metabolism by induction of low-grade inflammation. IL-17 also mediates immune responses by triggering the production of other proinflammatory cytokines, such as IL-6 and tumor necrosis factor-α (TNF-α), which is involved in the pathogenesis of insulin resistance. Interestingly, it has been reported that chronic infusion of angiotensin II increased the production of IL-17 from circulating T cells, thereby promoting angiotensin II–induced hypertension and vascular dysfunction. Therefore, we hypothesized that low-grade inflammation induced by IL-17 might contribute to insulin resistance induced by angiotensin II via AT1 receptor stimulation. These results led us to examine the possibility that IL-17 may play a pivotal role in the pathogenesis of angiotensin II–mediated insulin resistance.

Methods

All of the procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and reviewed and approved by the animal studies committee of Ehime University.
Animals
Eight-week-old adult male C57BL/6J and KK-Ay mice (CLEA, Tokyo, Japan) were used in this study. KK-Ay mice were originally developed by Nishimura by crossing the KK mouse with the Ay mouse (C57BL/6J-Ay). C57BL/6J mice are generally used as non-diabetic controls. Therefore, we used C57BL/6J mice as a control for KK-Ay. They were housed in an air-conditioned room at 25°C with a 12-hour light/12-hour dark cycle. They were given a standard diet (MF, Oriental Yeast, Tokyo, Japan) and water ad libitum. An AT1 receptor blocker, telmisartan, was provided by Boehringer Ingelheim (Ingelheim, Germany). Rat IgG3a, isotype control and antimouse neutralized IL-17 antibody (IL-17-nAb) were purchased from R&D Systems, Inc (Minneapolis, MN). KK-Ay mice were administered control-IgG3a and IL-17-nAb 5 times at a dose of 100 μg every second day by IP injection, as described previously with minor modification. Some KK-Ay mice were treated with telmisartan (1 mg/kg per day), hydralazine (250 mg/L), or IL-17-nAb (7 times at a dose of 100 μg every second day by IP injection). Systolic blood pressure was measured by the tail-cuff method (MK-2000ST, Muromachi Kikai Co, Ltd, Tokyo, Japan), as described previously.

Glucose Tolerance and Insulin Tolerance Tests
Oral glucose tolerance test (OGTT) was performed after 16 hours of overnight fasting. Glucose solution (2 g/kg) was administered orally, and a small amount of blood was obtained from the orbital sinus or tail vein without anesthesia at 0, 30, 60, and 120 minutes. For insulin tolerance test (ITT), mice were given an IP injection of 0.5 U/kg of insulin solution (NovoRapid, Novo Nordisk, Bagsvaerd, Denmark) after 4 hours of fasting. Blood glucose level was determined by glucose dehydrogenase pyrroloquinolinequinone method (Freestyle, Nipro, Osaka, Japan). Serum insulin level was measured by ELISA (Ultra Sensitive Rat Insulin kit, Morinaga Institute of Biological Science, Kanagawa, Japan).

Measurement of Serum IL-17, Insulin, Adiponectin, and TNF-α Concentrations
Blood samples were obtained from the inferior vena cava after 16 hours of fasting. Serum concentrations of IL-17 (Quantikine Mouse IL-17 Immunoassay, R&D Systems, Inc), insulin (Ultra Sensitive Rat Insulin kit, Morinaga Institute of Biological Science), adiponectin (Mouse/Rat High Molecular Weight Adiponectin ELISA kit, AKMAN-011, Shibayagi, Gunma, Japan), and TNF-α (Mouse TNF-α ELISA kit, AKMTN-011, Shibayagi) were measured using commercially available kits.

Measurement of Rate Constant of Net Tissue Uptake of 2-[3H]deoxy-D-Glucose
Uptake of 2-[3H]deoxy-D-glucose (2-[3H]DG) in peripheral tissues was measured as reported previously. Interscalp and subcutaneous white adipose tissue and skeletal muscles (extensor digitorum longus and soleus) were rapidly dissected and weighed. The rate constant of net tissue uptake of 2-[3H]DG was calculated as described previously.

Quantitative RT-PCR
Real-time quantitative RT-PCR was performed with a SYBR Premix Ex Taq (Takara Bio Inc). mRNAs were prepared from epididymal and retroperitoneal white adipose tissue after treatment with IgG3a and IL-17-nAb. PCR primers were as follows: 5’-CGGTGGACAA-3’/H11032 (forward) and 5’-CGAGATTTCCTT-3’ (reverse) for GAPDH; 5’-CGACGATTTCTCT-3’ (forward) and 5’-TTGGTCTTACACAGCAAACTC-3’ (reverse) for CGGTGGACAA-3’/H11032; 5’-CAGC-3’ (reverse) for CCAAT enhancer-binding protein-α, 5’-AACACCGAGATTTCCTT-3’ (forward) and 5’-ACATCTTCACCACACAACGACCCGCCCGT-3’ (reverse) for adipocyte protein 2, and 5’-ATGTAGGGCATGAGTCACAC-3’ (forward) and 5’-TGCGACTTCAACAGCAAACTC-3’ (reverse) for GAPDH.

Phosphorylation of Akt (Thr308)
Total proteins were prepared from soleus muscles with or without insulin treatment. After 16 hours of overnight fasting, the soleus muscle of C57BL/6J and KK-Ay mice was rapidly dissected. Soleus muscles of some mice were prepared at 30 minutes after treatment with IP injection of 1 IU/kg of insulin. Proteins were subjected to SDS-PAGE and immunoblotted with an antibody against phosphorylated Akt (Phospho-Akt Pathway Antibody Sampler kit, Cell Signaling Technology, Inc). The bands of proteins were visualized with an enhanced chemiluminescence system (GE Healthcare). Densitometric analysis was performed using National Institutes of Health image software.

Statistical Analysis
All of the values are expressed as mean ± SEM in the text and Figure. Data were evaluated by ANOVA followed by post hoc analysis for multiple comparisons. A difference with P<0.05 was considered significant.

Results
Blockade of IL-17 Ameliorated Insulin Resistance in KK-Ay Mice
The serum level of IL-17 was significantly higher in KK-Ay mice than in C57BL/6J mice (Figure 1A). We studied whether the higher level was related to insulin resistance induced by administration of mouse IL-17-nAb. Administration of IL-17-nAb did not change body weight, systolic blood pressure, or food intake compared with those in control KK-Ay mice. Blood glucose and serum insulin concentrations were higher in KK-Ay mice. Treatment with IL-17-nAb significantly reduced blood glucose level in the fed condition compared with that in vehicle-treated KK-Ay mice, whereas IgG3a treatment did not influence blood glucose concentration (Figure 1B). In contrast, treatment with IL-17-nAb did not change serum insulin level in KK-Ay mice (Figure 1C). In OGTT, the basal blood glucose concentration after 16 hours of fasting did not differ between each group, whereas the peak of the glucose rise in response to a glucose load was lower, and the decrease of blood glucose concentration was faster in mice treated with IL-17-nAb compared with control IgG3a-treated mice (Figure 2A). In contrast, there was no significant difference in serum insulin level among these groups (Figure 2B). In ITT, the decrease of blood glucose concentration after insulin injection was further enhanced in the IL-17-nAb–treated group (Figure 2C). Administration of IL-17-nAb significantly increased 2-[3H]DG uptake in skeletal muscle (extensor digitorum longus and soleus) with or without insulin stimulation (Figure 3A) but not in adipose tissue (Figure 3B). Consistent with this result, we observed that the insulin-mediated increase in phosphorylation of Akt (Thr308) in soleus muscles of KK-Ay mice was enhanced by administration of IL-17-nAb (Figure 3C).

Blockade of IL-17 Ameliorated Adipocytokine Dysregulation in KK-Ay Mice
Consistent with previous results, lower serum adiponectin and higher TNF-α levels were observed in KK-Ay mice.
adiponectin: 9.1±1.0 μg/mL versus 21.4±1.7 μg/mL, P<0.01, TNF-α: 904±95 pg/mL versus 48±40 pg/mL, P<0.01, respectively). We observed no significant morphological changes, such as adipocyte size, after IL-17-nAb treatment in KK-Ay mice (see Supplemental Figure, available online at http://hyper.ahajournals.org); however, IL-17-nAb treatment increased serum adiponectin and decreased TNF-α levels compared with those in vehicle-treated mice, whereas control-IgG2A treatment did not influence them (Figure 4A).

Moreover, mRNA expression of adiponectin and adipocyte-differentiation markers, such as peroxisome proliferator-activated receptor-γ, CCAAT enhancer-binding protein-α, and adipocyte protein 2, was enhanced in mice treated with anti-IL-17-nAb compared with vehicle-treated and control IgG2A-treated mice (Figure 4B).

**Role of IL-17 in AT1 Receptor-Mediated Insulin Resistance**

We examined the pathophysiological relevance of the involvement of possible cross-talk of the renin-angiotensin system and low-grade inflammation via IL-17 in terms of insulin resistance. Administration of an ARB, telmisartan (1 mg/kg per day) or losartan (1 mg/kg per day), decreased serum IL-17 level in KK-Ay mice (KK-Ay 79.0±11.5 pg/mL, KK-Ay+Tel 23.9±11.5 pg/mL, and KK-Ay+Los 29.4±5.9 pg/mL, respectively; Figure 5A). This dose of telmisartan did not affect baseline blood pressure in the KK-Ay or C57BL/6J mice. We also demonstrated that administration of angiotensin II (1.44 mg/kg per minute) via osmotic minipump for 2 weeks increased the serum IL-17 level in C57BL/6J mice (Figure 5A). This increase in IL-17

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Serum interleukin (IL) 17 level (A) and effects of administration of mouse neutralized-IL-17 antibody (IL-17-nAb) on glucose metabolism in KK-Ay mice. A, Serum IL-17 level in male C57BL/6J and KK-Ay mice was measured by ELISA (n=7–10 per group). Data are expressed as mean±SEM. *P<0.01 vs C57BL/6J. B, Effect of mouse IL-17-nAb administration on blood glucose and serum insulin concentrations in KK-Ay mice. *P<0.01 vs C57BL/6J; †P<0.01 vs KK-Ay control IgG2A.

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Effect of administration of mouse neutralized-IL-17 antibody (interleukin-17-nAb) on oral glucose tolerance test (OGTT; A), serum insulin level (B), and insulin tolerance test (ITT; C) in KK-Ay mice. Male KK-Ay mice were administered control-IgG2A and IL-17-nAb 5 times at a dose of 100 μg every second day by IP injection. OGTT and ITT were performed after fasting for 16 hours and 4 hours, respectively (n=8–10 per group). Data are expressed as mean±SEM. *P<0.01 C57BL/6J vs KK-Ay control-IgG2A; †P<0.01 vs KK-Ay control IgG2A.
was significantly attenuated by telmisartan but not by hydralazine (250 mg/L in drink solution), at doses that decreased systolic blood pressure to a similar level (Figure 5B). In OGTT, the peak of blood glucose concentration was increased by angiotensin II treatment, and this increase was attenuated by telmisartan and IL-17-nAb but not by hydralazine (Figure 5C). In ITT, the decrease of blood glucose concentration in response to insulin injection was impaired by angiotensin II treatment, and administration of telmisartan and IL-17-nAb enhanced the effect of insulin to lower the blood glucose level (Figure 5D). Hydralazine treatment did not affect the glucose concentration after insulin injection.

**Discussion**

It has been reported that plasma IL-17 concentration is higher in human obese patients, who are closely associated with numerous inflammatory conditions and insulin resistance. Obesity has been reported to be associated with enhanced IL-17 expression and increased severity of inflammation in IL-17–dependent mouse models. In addition, Jagannathan-Bogdan et al. demonstrated that blood obtained from T2DM patients showed increased Th17 cells and elevated activation of Th17 signature genes. Peripheral blood mononuclear cells from T2DM patients also secreted higher levels of IL-17 in response to T-cell stimuli, such as phytohemagglutinin and anti-CD3+anti-CD28 compared with those from nondiabetes subjects. Consistent with these findings, we demonstrated in this article that serum IL-17 is higher in diabetic KK-Ay mice than in C57BL and IL-17 neutralization by antibodyameliorated glucose intolerance in KK-Ay mice, with an increase in 2-[3H]DG uptake in skeletal muscle. In white adipose tissue, treatment with IL-17-nAb increased the expression of adipocyte differentiation markers and adiponectin. These results suggest that IL-17 could play pivotal roles in the pathogenesis of insulin resistance in T2DM. Moreover, we demonstrated that treatment with telmisartan or losartan decreased serum IL-17 level in KK-Ay mice and attenuated angiotensin II–induced insulin resistance, suggesting the possibility that cross-talk of IL-17 and AT1 receptor stimulation may regulate insulin resistance. Although telmisartan is reported to have a partial agonistic effect of peroxisome proliferator-activated receptor-γ, the reduction of IL-17 level in KK-Ay by treatment with telmisartan in this study is induced mainly by a blockade of AT1 receptor.

It is well known that chronic inflammation impairs glucose uptake in peripheral tissues, such as skeletal muscle, and plays an important role in the pathogenesis of insulin resistance. To our knowledge, there is no report concerning the regulation of distinct glucose transporters by IL-17; however, we observed that treatment with IL-17-nAb significantly increased 2-[3H]DG uptake in skeletal muscle of KK-Ay mice with enhanced phosphorylation of Akt (Thr308) but not in adipose tissue. Adipose tissue is also another important target in considering the role of IL-17 in the pathogenesis of insulin resistance. Therefore, the possible effects of higher IL-17 in serum of KK-Ay mice on adipose tissue have to be
addressed. We observed no significant morphological changes, such as adipocyte size, after IL-17-nAb treatment in KK-Ay mice; however, IL-17-nAb treatment increased serum adiponectin concentration, decreased serum TNF-α level, and enhanced adipocyte-differentiation markers. Therefore, we speculate that IL-17-nAb treatment improves functional change of adipose tissue, such as adiponectin release by anti-inflammation, but did not affect adipocyte size in short-time treatment. We observed that IL-17-nAb treatment increased serum adiponectin concentration and decreased serum TNF-α level. Inflammatory cytokines regulate the differentiation of adipocytes and their function, resulting in worsening of insulin resistance. For instance, TNF-α inhibits adipogenesis by preventing the induction of peroxisome proliferator-activated receptor-γ and CCAAT enhancer-binding protein-α (C/EBPα), and adipocyte protein 2 (aP2) in adipose tissue (epididymal [Epi] and retroperitoneal [Retro] white adipose tissues) of KK-Ay treated with control IgG2A or IL-17-nAb. mRNA expression of adiponectin and adipocyte-differentiation markers, such as PPARγ, C/EBPα, and aP2, was enhanced in KK-Ay mice treated with anti–IL-17-nAb compared with vehicle-treated and control IgG2A-treated KK-Ay mice. Data are expressed as mean±SEM. *P<0.05 vs KK-Ay control IgG2A.

Figure 4. A, Effect of mouse neutralized-IL-17 antibody (interleukin-17-nAb) administration on serum levels of adiponectin and tumor necrosis factor (TNF)-α (n=7 per group) in KK-Ay mice. Data are expressed as mean±SEM. *P<0.05 vs KK-Ay control IgG2A. B, mRNA expressions of adiponectin, peroxisome proliferator-activated receptor (PPARγ), C/EBPα, and adipocyte protein 2 (aP2) in adipose tissue (epididymal [Epi] and retroperitoneal [Retro] white adipose tissues) of KK-Ay treated with control IgG2A or IL-17-nAb. mRNA expression of adiponectin and adipocyte-differentiation markers, such as PPARγ, C/EBPα, and aP2, was enhanced in KK-Ay mice treated with anti–IL-17-nAb compared with vehicle-treated and control IgG2A-treated KK-Ay mice. Data are expressed as mean±SEM. *P<0.05 vs KK-Ay control IgG2A.

hepatic glucose production. Moreover, OGTT and ITT data in Figure 2 also support that IL-17 has a specific effect on insulin-mediated skeletal muscle glucose uptake. These results, including our results in this study, suggest that IL-17 blockade enhances adipocyte differentiation and improved their function, leading to improvement of insulin resistance in skeletal muscle. Further investigation concerning the regulation of distinct glucose transporters in skeletal muscle by IL-17 using in vitro experiments may be helpful to know the detailed function of IL-17.

We also demonstrated that treatment with telmisartan decreased serum IL-17 level in diabetic KK-Ay mice. Moreover, we showed that chronic angiotensin II infusion increased serum IL-17 concentration in C57BL/6J mice with glucose intolerance, which was ameliorated by lowering IL-17 level with telmisartan treatment. Interestingly, IL-17-nAb reduced glucose level but did not affect blood pressure in angiotensin II–treated mice, whereas telmisartan reduced both glucose level and blood pressure. These results indicate that skeletal muscle insulin resistance is not causally related to the hypertensive actions of angiotensin II, and angiotensin-induced IL-17 upregulation may further enhance insulin resistance independent of hypertensive action. Recently, the association between the effect of angiotensin II and Th17 response has been highlighted. Madhur et al showed that IL-17 production from circulating T cells of C57BL/6J mice was increased >5-fold by angiotensin II treatment, together
with hypertension and vascular dysfunction. In addition, another recent study demonstrated that an angiotensin-converting enzyme inhibitor or ARB modulated Th1 and Th17 responses in experimental autoimmune encephalomyelitis, which is an animal model of multiple sclerosis.13 We investigated Th17 cell number using flow cytometry; however, no significant difference was observed in mice with or without telmisartan (data not shown), indicating that angiotensin II may modulate Th17 response and increase IL-17 production. These results, including our results, suggest that the interaction between angiotensin II and the Th17 axis may have potential as a novel therapeutic target in the pathogenesis of AT1 receptor–induced insulin resistance.

A persistent inflammatory process with an increase in proinflammatory cytokines and chemokines leads to adipose tissue remodeling in obese subjects and obesity-related complications, such as diabetes mellitus.15 It has been reported that angiotensinogen gene expression in hypertrophied adipose tissue of not only obese mice17 but also obese humans was increased compared with that in nonobese subjects. In addition, Engeli et al18 have demonstrated that circulating levels of renin-angiotensin system components, such as angiotensinogen, renin, and aldosterone, were significantly higher in obese women than in lean women, suggesting that the systemic renin-angiotensin system is enhanced in obesity. Similarly, cross-sectional and prospective studies have shown that elevated inflammatory factors, including IL-17, were observed in obesity and T2DM.4–8,29 IL-17 mediates the immune response by triggering the production of other proinflammatory cytokines.16 In view of this, IL-17 responses may contribute to renin-angiotensin system activation and play an important role in the pathogenesis of AT1 receptor–induced insulin resistance.

**Perspectives**

Our results demonstrate a new aspect of the effect of ARB in insulin resistance and an important role of IL-17 in the pathogenesis of AT1 receptor-induced insulin resistance. Blockade of low-grade inflammation induced by IL-17 responses may have potential as a novel therapeutic target in insulin resistance of obesity and T2DM.

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**Disclosures**

None.
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Roles of interleukin-17 in angiotensin II type 1 receptor-mediated insulin resistance

Running title: Interleukin-17 and insulin resistance

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Figure S1
Figure S1 Legend:

**Figure S1.** (A) Representative photos of epididymal and retroperitoneal white adipose tissue in KK-Ay treated with neutralized-IL-17 antibody (IL-17-nAb) or control-IgG2A. (B) Histogram analysis of adipocyte number per mm² in these photos.  Epi; epididymal and Retro; retroperitoneal.