Obesity-related chronic kidney disease is associated with spleen-derived IL-10


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Keywords: chronic kidney disease, IL-10, inflammation, obesity, spleen

ABSTRACT

Background. Obesity is associated with systemic low-grade inflammation and is a risk factor for chronic kidney disease (CKD), but the molecular mechanism remains uncertain. We noticed spleen-derived interleukin (IL)-10 because it is observed that obesity reduces several cytokines in the spleen.

Methods. We examined whether spleen-derived IL-10 regulates CKD caused by a high-fat diet (HF)-induced obesity as follows: (i) male mice were fed with HF (60% fat) during 8 weeks and IL-10 induction from the spleen was examined, (ii) glomerular hypertrophy, fibrosis, inflammatory responses in the kidney and systolic blood pressure (SBP) were evaluated in splenectomy (SPX)-treated mice fed HF, (iii) exogenous IL-10 was systemically administered to HF-induced obese mice and the alteration of obesity-induced pathogenesis caused by IL-10 treatment was assessed. (iv) IL-10 knockout (IL-10KO) mice were treated with SPX and glomerular hypertrophy, fibrosis and the inflammatory condition in the kidney and SBP were also investigated.
Results. Obesity decreased serum levels of only IL-10, an anti-inflammatory cytokine even though pro- and anti-inflammatory cytokine expression in the spleen was significantly lower in the obese group. SPX aggravated HF-induced inflammatory responses in the kidney and hypertension. These HF-induced alterations were inhibited by systemically administered IL-10. Moreover, SPX had little effect on inflammatory responses and SBP in the kidney of IL-10KO mice.

Conclusions. We suggest that obesity reduces IL-10 induction from the spleen, and spleen-derived IL-10 may protect against the development of CKD induced by obesity.

INTRODUCTION

Obesity leads to the infiltration of fat in multiple organs, including the liver, heart and kidneys. Obese conditions increase fatty acid flux to the liver and pancreas [1, 2]. Infiltration of the liver by fat, or nonalcoholic fatty liver disease (NAFLD), is a major form of chronic liver disease in adults and children [3]. Recent studies have reported that high-fat diet (HF)-fed mice show several types of renal damage, and obesity is believed to have a direct role in the pathogenesis of chronic kidney disease (CKD). Pathological studies have demonstrated that subjects with severe obesity develop proteinuria with pathological findings of podocyte hypertrophy, mesangial expansion, glomerular enlargement, and focal segmental diabetes and hypertension [4, 5]. Obesity is a common risk factor for CKD and NAFLD, and it is not surprising that the two conditions are associated [6, 7].

The spleen is the largest lymphoid organ in the body and plays an important role in the host immune function. Obese rats show decreased expression of pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) in the spleen [8]. In contrast, IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of pro-inflammatory cytokines. It is synthesized by several cell types within multiple organs, including the spleen. Large amounts of IL-10 are produced by activated B cells, which mature in the marginal zone of the spleen. New evidence has shown that IL-10-producing B cells play a regulatory role in suppressing harmful immune responses [9]. In fact, low IL-10 production capacity has been demonstrated in obesity [10, 11]. Based on these findings, obesity is hypothesized to suppress IL-10 synthesis, resulting in chronic kidney inflammation. Our data demonstrate that obesity reduced IL-10 expression in the spleen and that spleen-derived IL-10 protected against inflammatory responses induced by obesity in the kidney.

MATERIALS AND METHODS

Animals

Male C57Bl/6J mice (wild-type mice, 22–25 g; KBT Oriental, Japan) and IL-10-deficient mice (IL-10KO mice, 002251-B6.129P2-H10tm1Cgn/J, a gift from Sandy Morse, The Jackson Laboratory, Bar Harbor, ME, USA) were housed in a room at Oita University under a 12/12-h light/dark cycle with lights on from 07:00 to 19:00. IL-10KO mice maintained at our university were used for backcrossing. Polymerase chain reaction primers of 5′-CCACACGGTCACCTAA-A-3′ (mutant forward), 5′-GGTTATATTGCTTCGCGGTGT-3′ (wild-type reverse) and 5′-CTTGCACTACAAAGGCCACA-3′ (common) were used for genotyping. All studies were conducted in accordance with Oita University guidelines, based on the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Experimental protocol

Experiment 1. Wild-type mice were assigned to one of two groups (n = 6 in each group) as follows: Group 1, mice were fed standard chow (standard: 20% fat, 56% carbohydrate, 24% protein; Diet Research, New Brunswick, NJ, USA) for 8 weeks and then subjected to sham operation (Sham); Group 2, mice were fed standard chow for 8 weeks and then underwent splenectomy (SPX). Anesthesia was then induced by intraperitoneal injection of sodium pentobarbital (50 mg/kg), the abdominal cavity was opened and the spleen was carefully removed. The abdomen was opened, but the spleen was not removed in the sham group.

Experiment 2. Wild-type mice were assigned to one of four groups (n = 6 in each group) as follows: Group 1, mice were fed standard chow for 8 weeks after sham operation and administered mouse serum albumin (m-albumin); Group 2, mice were fed a HF (60% fat, 20% carbohydrate, 20% protein; Diet Research) for 8 weeks after sham operation and then given m-albumin; Group 3, mice were fed HF for 8 weeks after SPX and then given m-albumin; Group 4, mice were fed HF for 8 weeks after sham operation and then given recombinant mouse IL-10 (r-IL-10, 0.5 ng/day; Wako Chemicals, Osaka, Japan).

Experiment 3. Wild-type and IL-10KO mice were assigned to one of three groups (n = 6 per each group) as follows: Group 1, mice were fed HF for 8 weeks after sham operation and administered m-albumin; Group 2, mice were fed HF for 8 weeks after SPX and administered m-albumin; Group 3, mice were fed HF for 8 weeks after SPX and administered r-IL-10 (0.5 ng/day; Wako Chemicals). Body weight was measured between 17:00 and 18:00 h. All mice were housed for an additional 4 weeks after completion of the interventions.

Cytokine levels in the spleen, kidney and serum

Commercial enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen, Carlsbad, CA, USA) were used to measure the TNF-α, IL-1β, monocyte chemotactic protein-1 (MCP-1), IL-10, IL-4 and IL-13 levels in the spleen, kidney and serum. Neutrophil gelatinase-associated lipocalin (NGAL), an early biomarker of acute kidney injury, levels in the kidney were also measured using the NGAL ELISA kit (BioPorto, Gentofte, Denmark). Protein concentrations in each organ were analyzed using the Lowry method.
Western blotting

Frozen tissue preparations were homogenized with sample buffer, centrifuged and boiled. Total protein concentration of tissues was quantified using the Bradford method. Equal amounts of total protein were loaded on 8% sodium dodecyl sulfate-polyacrylamide gels for electrophoresis and then electrophoretically transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% nonfat milk for 1 h, incubated overnight with primary antibodies at 4°C and then incubated with a secondary antibody for 1 h at room temperature. The primary antibody solutions consisted of polyclonal antisera with specificity for rabbit desmin and nephrin (Acris Antibodies, Inc., San Diego, CA, USA). Desmin and nephrin were detected by enhanced chemiluminescence (Amersham Life Sciences, Fairfield, CT, USA) and quantified using Quantity One® imaging software (Bio-Rad).

Histological and immunohistochemical analyses

Kidney samples were fixed in 4% buffered paraformaldehyde, embedded in paraffin, sectioned and deparaffinized in xylene. Samples were examined with periodic acid-Schiff (PAS) and Mallory-Azan reagents.

For immunohistochemical staining of F4/80 and NGAL, 5-µm-thick frozen kidney sections were incubated overnight at 4°C with rabbit anti-mouse F4/80 (Acris Antibodies GmbH, Herford, Germany). Slides were subsequently washed with phosphate-buffered saline and incubated with biotin-conjugated goat anti-rabbit IgG (ABC reagent, Vector Laboratories, Inc., Seattle, WA, USA) and then incubated with biotin-conjugated rabbit anti-goat IgG or goat anti-rabbit IgG (ABC reagent, Vector Laboratories). Samples were visualized with rhodamine-conjugated or fluorescein isothiocyanate-conjugated streptavidin. Additionally, normal goat or rabbit serum was used instead of the aforementioned antibodies, and further incubation with a secondary antibody was performed as a negative control, which resulted in no staining.

Glomerular mesangial proliferation

Mesangial cells were counted on 10 arbitrarily selected glomeruli on PAS-stained kidney sections. Glomeruli with a diameter of 100–150 µm were evaluated.

Fibrosis in the kidney

Kidney sections were subjected to Mallory-Azan staining. The mean fibrotic area, indicated by a blue color after Mallory-Azan staining, in 10 glomerular areas per animal was quantified using a 0.02-mm² graticulate eyepiece.

Table 1. Effects of HF feeding on splenic and serum levels of pro- and anti-inflammatory cytokines

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Standard (pg/mg protein)</th>
<th>HF (pg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>105.2 ± 6.5</td>
<td>52.8 ± 7.3*</td>
</tr>
<tr>
<td>IL-1β</td>
<td>42.8 ± 5.1</td>
<td>25.7 ± 3.1*</td>
</tr>
<tr>
<td>MCP-1</td>
<td>16.9 ± 1.7</td>
<td>9.0 ± 1.4*</td>
</tr>
<tr>
<td>IL-4</td>
<td>24.5 ± 3.7</td>
<td>16.7 ± 3.5*</td>
</tr>
<tr>
<td>IL-10</td>
<td>20.2 ± 2.5</td>
<td>9.3 ± 1.1*</td>
</tr>
<tr>
<td>IL-13</td>
<td>9.7 ± 1.2</td>
<td>7.1 ± 1.4*</td>
</tr>
</tbody>
</table>

Table 2. Effects of SPX on serum cystatin C level and SBP

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum cystatin C level (ng/mL)</th>
<th>SBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard-sham</td>
<td>1.18 ± 0.11</td>
<td>101.4 ± 2.8</td>
</tr>
<tr>
<td>Standard-SPX</td>
<td>1.98 ± 0.14*</td>
<td>108.7 ± 1.6*</td>
</tr>
</tbody>
</table>

*P < 0.05 versus standard-sham group. Treatment groups: standard-sham, fed a standard chow and given a sham operation; standard-SPX, fed a standard chow and given an SPX.
evaluated as a percentage of the field using Mac Scope version 6.02 (Mitani Shoji, Fukui, Japan).

**Serum cystatin C level**

The serum cystatin C level was measured with a cystatin C ELISA kit (Quantikine, R&D System Inc.).

**Measurement of systolic blood pressure**

Systolic blood pressure (SBP) was measured in conscious mice by the tail-cuff method (Softron, Tokyo, Japan). On each day of SBP determination, 10 measurements were obtained and averaged for each mouse.

**Statistics**

Results were expressed as mean ± standard error of the mean. Statistical tests included two-tailed Student’s t-test and two-way analysis of variance followed by Scheffe’s test for post hoc comparison. For all tests, the level of significance was set at P < 0.05.

**RESULTS**

**HF feeding decreases serum levels of IL-10, but not IL-1β or MCP-1**

Even though pro-inflammatory (TNF-α, IL-1β, and MCP-1) and anti-inflammatory (IL-4, IL-10, and IL-13) cytokine expression in the spleen was significantly lower, the serum level of only IL-10 was significantly lower in the HF group compared with the standard group (Table 1). When splenic cytokine expression was downregulated by HF feeding, the serum cytokine levels, except IL-10, were probably maintained by organs other than the spleen. However, the low serum IL-10 levels suggest that large amounts of serum IL-10 are derived from the spleen.

**SPX causes renal dysfunction and elevates SBP**

We investigated whether SPX contributes to renal function and SBP. SPX treatment elevated serum cystatin C levels and SBP, compared with sham treatment (Table 2).
SPX accelerates renal damage and promotes inflammation in the kidney. Next, we investigated whether SPX resulted in inflammation in the kidney. The PAS-stained renal pictures obtained in each group are shown in Figure 1A. Histological examination of the kidney revealed glomerular hypertrophy and expansion of the mesangial area in the SPX group compared with the sham group (Figure 1A and B). To examine renal injury, due to SPX, nephrin and desmin expression were evaluated qualitatively by immunofluorescence in kidney sections from each group (Figure 1A) and quantitatively by western blot (Figure 1C and D). SPX treatment significantly reduced glomerular nephrin expression and elevated desmin expression compared with sham treatment. Glomerular fibrosis was greater in the SPX group than in the sham-treated group (Figure 1A and E). Moreover, we examined the staining of kidney tissues for F4/80 in each group to evaluate changes in macrophage infiltration after SPX. The macrophage marker F4/80 staining level in glomerular and tubulointerstitial areas was increased (Figure 1A and F) in the SPX-treated group compared with the sham-treated group. Furthermore, SPX also increased pro-inflammatory cytokines such as TNF-α, IL-1β and MCP-1, but decreased anti-inflammatory cytokine, IL-10 levels, but not IL-4 and IL-13 (Figure 1G) in the kidney compared with sham treatment. We measured NGAL protein levels in the kidneys, but there was no difference between sham and SPX groups (Figure 1H and I).

**IL-10 treatment inhibits the HF-induced glomerular injury**

The effect of IL-10 on the HF-induced alterations in glomeruli was examined. Compared with standard feeding, HF feeding induced glomerular hypertrophy and hyperplasia of mesangial cells (Figure 2A and B) and led to decreased nephrin (Figure 2A and C) and increased desmin (Figure 2A and D) expression, suggesting that HF feeding induces renal injury. These alterations were promoted in the HF-fed SPX group to a significantly greater degree than in the HF-fed sham group (Figure 2). However, IL-10 treatment attenuated the progression of glomerular fibrosis and the alterations of nephrin and desmin expression induced by HF feeding (Figure 2).

**IL-10 treatment diminishes HF-induced macrophage infiltration and fibrosis in the glomerulus, and also attenuates elevation of SBP**

Interestingly, compared with standard feeding, HF feeding promoted F4/80-positive cell infiltration in glomerular as well as tubulointerstitial areas (Figure 3A and B) and fibrosis in

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**FIGURE 2:** The systemic administration of IL-10 suppresses HF-induced renal damage. (A) Representative PAS staining (left row), nephrin staining (middle row) and desmin staining (right row) in the glomerular area of kidney sections from each group. Scale bar = 100 µm. (B–D) Expansion of the mesangial area (B), nephrin expression (C) and desmin expression (D) in kidneys from each group (n = 6). *P < 0.05 versus standard-sham group, **P < 0.05 versus HF-sham group. Treatment groups: standard-sham, fed a standard chow, administered serum albumin and given a sham operation; HF-sham, fed a HF, administered serum albumin and given a sham operation; HF-SPX, fed a HF, administered serum albumin and given an SPX, HF-sham + IL-10, fed a HF, administered IL-10 and given a sham operation.
glomeruli (Figure 3A and C) and increased SBP (Figure 3D). SPX treatment further aggravated these alterations induced by HF feeding, and IL-10 treatment suppressed HF-induced changes in inflammatory responses and blood pressure (Figure 3).

**SPX alters the expression of pro- and anti-inflammatory cytokines in the kidney, elevates serum cystatin C levels and reduces the level of serum IL-10 more than the levels of other pro-inflammatory cytokines**

HF feeding increased pro-inflammatory and decreased anti-inflammatory cytokine levels except IL-4 and IL-13 in the kidney, compared with standard feeding (Figure 4A). In addition, serum cystatin C levels were higher in HF feeding, compared with standard feeding (Figure 4B). Furthermore, SPX accelerated these HF-induced changes, and IL-10 treatment suppressed the HF-induced alterations (Figure 4A and B). On the other hand, there was no significant difference in the renal NGAL level in all groups (Figure 4C and D). To clarify whether spleen-derived IL-10 is more relevant to inflammation than IL-10 produced locally in the kidney, we examined serum cytokine levels in the sham and SPX groups on standard and HF feeding. SPX decreased both pro- and anti-inflammatory cytokine levels in the serum, irrespective of standard or HF feeding (Figure 4E). However, SPX reduced the serum IL-10 level by approximately 60% in mice on standard and HF feeding, and the ratio of the decrease in IL-10 by SPX was greater than that of pro-inflammatory cytokines in mice on both standard and HF feeding (Figure 4F).

**SPX does not cause glomerular hypertrophy and injury in the IL-10-deficient mice**

The proliferation of mesangial cells and renal injury were greater in IL-10KO mice than in wild-type mice (Figure 5). SPX promoted hyperplasia of mesangial cells (Figure 5A and B) and renal injury (Figure 5A, C and D), compared with sham treatment in wild-type mice. On the other hand, these alterations were not found in IL-10KO mice (Figure 5). However, IL-10 treatment improved these alterations in both wild-type and IL-10KO mice.
SPX does not affect renal injury, including fibrosis, SBP or serum cystatin C levels in the kidneys of IL-10-deficient mice

The infiltration of F4/80-positive cells and glomerular fibrosis were greater in IL-10KO mice than in wild-type mice. SPX resulted in acceleration of F4/80-positive cell infiltration and fibrosis in the glomeruli, compared with sham treatment in wild-type mice (Figure 6A and B). However, these observations were not seen in IL-10KO mice. Furthermore, SPX-induced glomerular macrophage infiltration and fibrosis (Figure 6A and C), elevation of SBP and serum cystatin C level (Figure 6D and E) observed in wild-type mice were also not found in IL-10KO mice. Meanwhile, IL-10 treatment improved these alterations in both wild-type and IL-10KO mice (Figure 6).

SPX has no effect on pro-inflammatory responses in IL-10-deficient mice

SPX-induced acceleration of F4/80-positive cells in tubulointerstitial areas and increase of renal pro-inflammatory cytokine contents were not observed in IL-10KO mice (Figure 7A and B). Furthermore, IL-10 treatment also improved these alterations in both mice (Figure 7A and B). However, there was no difference in renal IL-4, IL-13 and NGAL levels among all groups (Figure 7B and D).

DISCUSSION

Obesity is associated with a low-grade, chronic, pro-inflammatory condition [12, 13]. However, the primary cause of obesity-induced inflammation is not well understood. Our previous study revealed that SPX aggravated obesity-induced inflammatory responses such as elevation of pro-inflammatory cytokines (TNF-α, IL-1β and MCP-1) and infiltration of F4/80-positive cells in the liver of wild-type mice, but not of IL-10KO mice [14]. This is the first study to systematically characterize the effect of a lack of IL-10 production in the spleen due to HF-induced obesity and SPX in mice, with respect to the kidney.

IL-10 is synthesized by several cell types in multiple organs, including the spleen. We noted spleen-derived IL-10 because...
the serum level of only IL-10, despite the significantly decreased expression of all cytokines in the spleens of HF-fed mice compared with standard-fed mice. This suggested that large amounts of serum IL-10 are derived from the spleen and that obesity reduces IL-10 secretion from the spleen. Our previous research showed that HF feeding compared with standard feeding, downregulated the expression of CD20, a surface molecule present on B cells, which play a large role in the immune response and produce IL-10 mainly in the spleen [15]. Moreover, splenocyte proliferation stimulated by T-cell and B-cell mitogens was significantly lower in obese subjects; thus, the functions of both T-cells and B-cells in the spleen may be impaired in obesity [16]. We hypothesized that the obesity-induced reduction in IL-10 synthesis in the spleen could lead to inflammatory responses in the kidneys and to metabolic disorders.

It is known that podocyte loss and dysfunction occur as the magnitude of glomerulosclerosis increases. Because podocytes serve as the final barrier against urinary loss in normal glomeruli, any change in podocyte structure or function may be intimately associated with proteinuria and consequent glomerular sclerosis [17]. It is possible that the loss of podocyte nephrin is the transition step from an obesity-related glomerular change to the pathogenesis of proteinuria [18]. Our data suggest that the obesity-induced reduction of nephrin expression was markedly accelerated by SPX and that IL-10 administration to HF-fed mice elevated nephrin expression to a level comparable with that in standard-fed mice. We also found that the obesity-induced increase in desmin, an intermediate filament protein and a specific and sensitive podocyte injury marker, was elevated in the glomeruli after SPX. However, HF-induced increase in desmin expression in the glomeruli was attenuated by IL-10 administration. Furthermore, the alterations of glomerular hypertrophy, fibrosis and hyperplasia of mesangial cells, SBP and serum cystatin C level that reflects severe renal damage were similar in all groups [19, 20]. These results would support our assertion that splenic IL-10 production is necessary to downregulate HF-induced pro-inflammatory response to renal damage.

To further clarify the influence of spleen-derived IL-10 in protection against inflammation in the kidney induced by SPX treatment, we examined whether IL-10 deficiency affected SPX-induced alterations in the kidneys using IL-10KO mice. The SPX-induced pro-inflammatory effects, acceleration of

**FIGURE 5:** SPX has little effect on renal damage in IL-10-deficient mice. (A) Representative PAS staining (left row), nephrin staining (middle row) and desmin staining (right row) in the glomerular area of kidney sections from each group (n = 6). Scale bar = 100 µm. (B–D) Expansion of mesangial area (B), nephrin expression (C) and desmin expression (D) in kidneys of each group (n = 6). *P < 0.05 versus sham (wild-type or IL-10KO) group, #P < 0.05 versus SPX (wild-type) group. Treatment groups: sham, fed a HF, administered serum albumin and given a sham operation; SPX, fed a HF, administered serum albumin and given an SPX; SPX + IL-10, fed a HF, administered r-IL-10 and given an SPX; Wild-type, wild-type mice; IL-10KO, IL-10-deficient mice.
hypertrophy and fibrosis in glomeruli, decrease of nephrin, increase of desmin expression in the kidneys and elevation of SBP as well as serum cystatin C levels observed in wild-type mice were not seen in IL-10KO mice. However, IL-10 treatment restored these alterations in the kidney in SPX-treated wild-type mice and IL-10KO mice. These results support the view that the obesity-induced reduction of spleen-derived IL-10 may result in renal damage. NGAL also has great promise as a biomarker of CKD including diabetic nephropathy [21, 22]. However, in this research, renal NGAL levels were not increased in the absence of spleen-derived IL-10, supporting the other study showing there is no difference in the whole kidney of streptozotocin (STZ) as a model of slowly progressive CKD and non-STZ mice. We suggest that NGAL might be useful as a marker of acute worsening of CKD because other research propose that induction of NGAL expression is a real-time indicator of acute renal injury [23]. Taken together, these data suggest that IL-10 originating in the spleen may prevent obesity-induced CKD.

In chronic inflammatory conditions, defective IL-10 synthesis contributes to increased pro-inflammatory cytokine levels [24]. We examined serum cytokine levels in the sham and SPX groups on standard and HF feeding. We found that the serum IL-10 level was reduced by more than half by SPX in mice on both standard and HF feeding, which induces a strong systemic inflammatory response. Simultaneously, SPX treatment also decreased the expression of IL-10 locally in the kidneys. In contrast, we also observed that SPX induced a maximum 40% decrease in serum pro-inflammatory cytokine levels, suggesting that pro-inflammatory cytokines are predominantly produced in other organs except the spleen, rather than anti-inflammatory cytokines such as IL-10, although both pro- and anti-inflammatory cytokines are produced in the spleen [8, 9]. Indeed, SPX eliminates both pro- and anti-inflammatory cytokine productions by the spleen. However, because SPX did not affect fat accumulation and inflammatory responses in the kidneys of IL-10-deficient mice, the SPX-induced alterations observed in the kidneys of wild-type mice were likely not due to the elimination of pro-inflammatory cytokines after SPX.

Macrophage infiltration in the kidney has an important role in the development and progression of renal diseases. HF feeding triggers the recruitment of macrophages, which release pro-inflammatory cytokines causing metabolic disorders including CKD [25]. Moreover, the balance between pro-inflammatory and anti-inflammatory cytokines determines the
inflammatory response and may mediate the progression of CKD [26]. Thus, the suppression of pro-inflammatory cytokines could retard or alleviate inflammation and improve CKD. In this study, SPX treatment increased pro-inflammatory cytokines, but decreased anti-inflammatory cytokine, IL-10 and elevated macrophage infiltration in the kidney. These responses were not found in IL-10KO mice. However, the supplementation of IL-10 to SPX-treated wild-type and IL-10KO mice was associated with the downregulation of pro-inflammatory cytokine production and macrophage infiltration in the kidney. We therefore considered the possibility that macrophage functions may be modulated by spleen-derived IL-10. This suggests that abnormally activated inflammation in the kidney may contribute to the lack of spleen-derived IL-10. On the other hand, we found that SPX treatment did not alter renal IL-4 and IL-13 expression in either wild-type or IL-10KO mice, which is compatible with other studies demonstrating that there is no difference in the expressions of both IL-4 and IL-13 between wild-type and IL-10KO mice [27] although the reason is unclear. To our knowledge, our report is the first demonstration that obesity-related CKD may be characterized by the promotion of pro-inflammatory pathways accompanied by diminished spleen-derived IL-10. In conclusion, we have demonstrated a critical role for spleen-derived IL-10 in the inflammation of kidneys in the obese state. Although additional work is needed to understand why obesity elicits an inflammatory response, our data indicate that a more comprehensive understanding of the interactions between obesity and the spleen may provide a therapeutic strategy for treating CKD.

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**CONFLICT OF INTEREST STATEMENT**

None declared.

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