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High IL-1 α production was induced in the WBN/Kob-*Lep^{fa}* type 2 diabetes mellitus rat model and inhibited by *Syphacia muris* infection

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Summary

The novel WBN/Kob-*Lep^{fa}* (*fa/fa*) congenic rat strain is considered a useful rat model of type 2 diabetes mellitus (T2DM). Accumulating findings suggest that low-grade inflammation is a causative factor in T2DM and that circulating levels of inflammatory cytokines are associated with insulin resistance. However, inflammatory cytokine profiles and their correlations with T2DM development/progression in *fa/fa* rats have not been studied. In this study, we found that the *fa/fa* rats had considerably high plasma levels of interleukin (IL)-1 α . Abundant cecal IL-1 α mRNA expression and cecal inflammation with infiltrating IL-1 α -producing macrophages was observed in *fa/fa* rats. Bone marrow derived macrophages from *fa/fa* rats expressed high levels of IL-1 α upon lipopolysaccharide stimulation. Furthermore, *Syphacia muris* infection, which delays the onset of T2DM, reduced both plasma and cecal IL-1 α levels in *fa/fa* rats. These results suggest that macrophage infiltration and IL-1 α secretion comprise an important part of T2DM development and that *S. muris* infection inhibits pro-inflammatory cytokine expression in *fa/fa* rats.

Keywords: IL-1 α ; macrophage; *Syphacia muris*; type 2 diabetes mellitus; WBN/Kob-*Lep^{fa}*

Introduction

Type 2 diabetes mellitus (T2DM) is a major international health problem with an increasing global prevalence (Frag et al., 2011; Zimmet et al., 2001). Approximately 95 % of patients with diabetes mellitus suffer from T2DM, which is characterized by insulin resistance and pancreatic β -cell dysfunction. Recent studies have demonstrated that chronic low-grade inflammation with elevated levels of pro-inflammatory cytokines contributes to the onset and progression of T2DM (Donath et al., 2011). For example, interleukin (IL)-1 β , a representative pro-inflammatory cytokine, is produced via the Nod-like receptor (NLR) family pyrin domain containing 3 (NLRP3) inflammasome pathway following activation by free fatty acids (Wen et al., 2011). IL-1 β leads macrophage recruitment and islet β -cell death by inducing upregulation of the pro-apoptotic

receptor Fas (Maedler et al., 2002). Anti-inflammatory approaches that target specific pro-inflammatory cytokine pathways are expected to be efficacious in the treatment or prevention of T2DM. Animal models of T2DM are needed to elucidate pathogenesis and conduct pharmacological studies of new candidates for therapeutic or preventive options. The WBN/Kob-*Lep^{fa}* (*fa/fa*) rat is a new congenic strain established by introducing the leptin receptor fatty gene (*Lp^{fa}*), a spontaneous mutation derived from Zucker-fatty rats that develop obesity and hyperinsulinemia consequent to leptin receptor dysfunction (Zucker, 1965; Chua et al., 1996), into the parental WBN/Kob rat genome (Akimoto et al., 2008). Previous reports revealed that *fa/fa* rats develop hyperglycemia at 9 weeks of age, evidenced by impaired glucose tolerance, insulin resistance, and dyslipidemia in a manner similar to human T2DM8 (Akimoto et al., 2008; Akimoto et al., 2012; Kaji et al., 2012; Okuno

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et al., 2013; Nagakubo *et al.*, 2014), thus suggesting that the *fa/fa* rat is useful as a new model of T2DM.

In a previous study, we demonstrated that infection with *Syphacia* (*S.*) *muris*, a nematode that targets rats, delayed the onset of hyperglycemia in *fa/fa* rats (Taira *et al.*, 2015). Furthermore, a global transcriptomic next-generation sequencing analysis of *S. muris* suggested that some genes expressed in *S. muris* might modify the host immune response; these genes include a gene involved in the regulation of macrophage activation via the Fc receptor signaling pathway (Okamoto *et al.*, 2015). Those studies suggested that *S. muris* infection might regulate the local production of cytokines and chemokines that promote the development of T2DM. However, the inflammatory cytokine profiles and their contributions to the development and progression of T2DM have not been investigated in *fa/fa* rats. The aim of present study, therefore, was to evaluate cytokine/chemokine profiles in *fa/fa* rats and compare them with profiles of control Wistar rats or *S. muris*-infected *fa/fa* rats. In addition, cells, organs or tissues, and/or tissues that produce inflammatory cytokines in *fa/fa* rats were also investigated.

Materials and Methods

Animals

Male WBN/Kob-*Lep^{fa}* (*fa/fa*) and Wistar rats (control) were obtained from SLC Inc. (Shizuoka, Japan). All rats were maintained under conventional conditions with a 12 h on/off light cycle, commercial diet, and water *ad libitum*. All animal studies were approved by the Azabu University Animal Research Ethics Committee (approval No. 150303-3). All animal procedures were performed under the experimental animal guidelines of Azabu University. Blood glucose levels of *fa/fa* rats were measured weekly.

Parasites and infection

S. muris infection was performed as described previously (Taira *et al.*, 2015). Briefly, *S. muris* eggs were harvested from the uterus of female worms obtained from the colons and rectums of experimentally infected male Wistar rats (Stahl, 1961). At 5 weeks of age, *fa/fa* rats were inoculated once via stomach tubes with either approximately 100 eggs/ml/rat or 1 ml/rat of distilled water (for uninfected group). *S. muris* infection was confirmed using cellophane tape to detect pinworm eggs in the perianal regions of rats.

Blood sampling

Blood samples were collected from the tail vein, and plasma samples were obtained by centrifuging heparinized blood. In some experiments we used Wistar rats' plasma purchased from SLC Inc. as a "control."

Cytokine and chemokine array

To measure plasma cytokine and chemokine levels, pooled plasma from 9 – 10-week-old *fa/fa* rats, *S. muris*-infected *fa/fa* rats, and control Wistar rats plasma (SLC Inc.) were used. Cytokine

profiles were analyzed using the Proteome Profiler™ Rat Cytokine Array Panel A (R&D Systems, Wiesbaden, Germany), which can identify 29 cytokines (Fig. 1A). All experiments were performed according to the manufacturer's instructions. Spots were visualized using Immobilon Western (Merck Millipore, Massachusetts, USA) and captured with an ImageQuant LAS-4000 (GE Healthcare, Buckinghamshire, UK). ImageQuant TL software (GE Healthcare) was used for the data analysis. Signals were normalized using internal controls included on the array.

Enzyme-linked immunosorbent assay (ELISA)

IL-1 α levels in plasma or bone marrow-derived macrophage (BMDM) culture supernatant were quantified using a Rat IL-1 α Platinum ELISA (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions.

Quantitative real-time PCR (RT-qPCR)

RT-qPCR of IL-1 α was performed as described previously (Okamoto *et al.*, 2009), using RNA from ceca isolated from 10-week-old *fa/fa* and *S. muris*-infected *fa/fa* rats and Wistar rats. Total RNA was extracted from each cecum using an RNeasy Mini kit (QIAGEN, Hilden, Germany). Single-stranded cDNA was synthesized from total RNA using the RT2 First Strand Kit (QIAGEN). IL-1 α mRNA expression was determined by RT-qPCR with the RT2 SYBR Premix and primer sets for IL-1 α (ref.seq. NM_017019.1) and β -actin (ref.seq. NM_031144.2) or 18s RNA (ref.seq. X01117.1); these reagents were purchased from QIAGEN and used according to the manufacturer's instructions. Relative target gene quantification was calculated according to the comparative CT method.

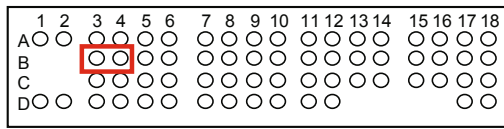
Histologic analysis

Cecal tissues were isolated from 12–16-week-old *fa/fa* rats and Wistar rats. Tissues were fixed in a 10 % neutral buffered formalin solution and embedded in paraffin blocks or, for frozen sections, in OCT compound (Sakura FineTek, Tokyo, Japan) after snap-freezing in liquid nitrogen as described previously (Nagakubo *et al.*, 2014; Aihara *et al.*, 2015). Histologic sections (4- μ m-thick) were stained with hematoxylin and eosin (HE).

Immunofluorescence analysis

To detect IL-1 α expression in the cecum, frozen cecal sections were incubated with a rabbit anti-IL-1 antibody (Santa Cruz Biotechnology, Dallas, TX, USA). Alexa Flour 488-conjugated goat anti-rabbit IgG (Invitrogen, Carlsbad, CA, USA) was used for secondary detection. To detect macrophages in the cecum, frozen sections were incubated with mouse anti-CD68 (Serotec, Oxford, UK). Alexa Flour 568-conjugated goat anti-mouse IgG (Invitrogen) was used for secondary detection. Sections were mounted in VECTASHIELD Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA, USA). Stained sections were examined and imaged using a fluorescence microscope (FSX100; Olympus, Tokyo, Japan).

A



 IL-1 α

coordinate	target	coordinate	target
A1, A2	control	C3, C4	IL-13
A3, A4	CINC-1	C5, C6	IL-17
A5, A6	CINC-2 α/β	C7, C8	CXCL10
A7, A8	CINC-3	C9, C10	LIX
A9, A10	CNTF	C11, C12	L-Selectin
A11, A12	CX3CL1	C13, C14	CXCL9
A13, A14	GM-CSF	C15, C16	CCL3
A15, A16	siCAM-1	C17, C18	CCL20
A17, A18	IFN- γ	D1, D2	control
B3, B4	IL-1 α	D3, D4	CCL5
B5, B6	IL-1 β	D5, D6	CXCL7
B7, B8	IL-1ra	D7, D8	TIMP-1
B9, B10	IL-2	D9, D10	TNF- α
B11, B12	IL-3	D11, D12	VEGF
B13, B14	IL-4	D17, D18	negative control
B15, B16	IL-6		
B17, B18	IL-10		

B

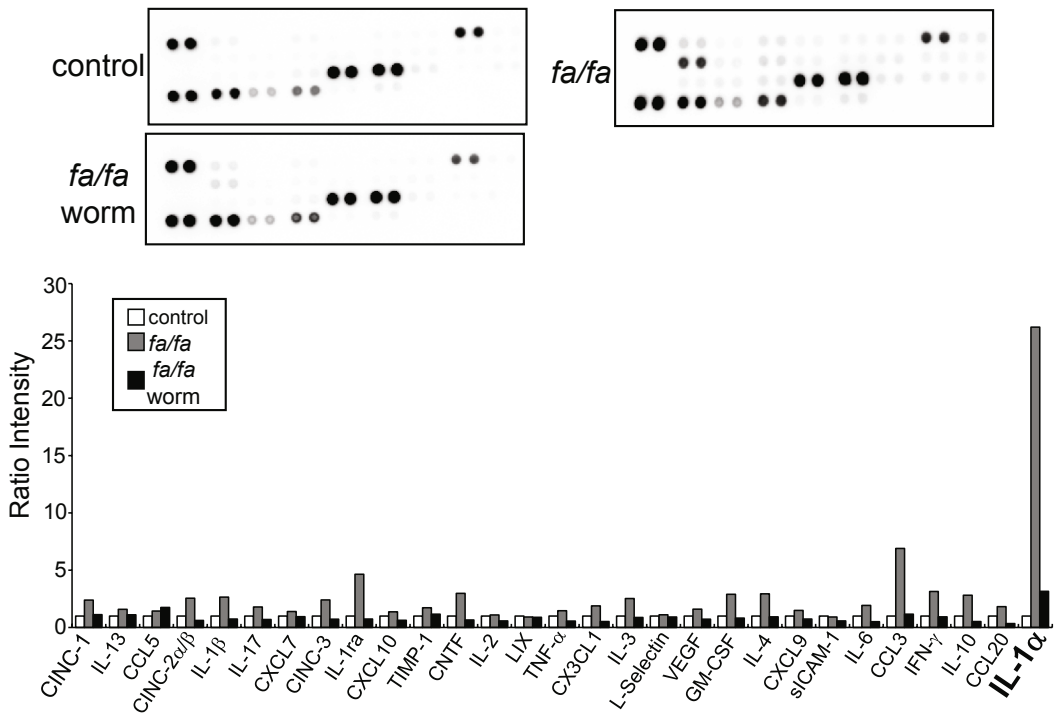


Fig. 1. Profile of circulating cytokines and chemokines in rats according to an array panel

(A) Rat cytokine/chemokine array panel coordinates. The IL-1 α coordinate is indicated by a box with red line.

(B) Plasma cytokines and chemokines from Wistar rats (control), *fa/fa* rats (*fa/fa*), and *Syphacia muris*-infected *fa/fa* rats (*fa/fa* worm) were measured using a rat cytokine/chemokine array panel. The signal intensity at each spot was normalized using internal controls included on the array, and relative levels of each cytokine/chemokine are presented as a graph.

Macrophage differentiation and stimulation

BMDMs were generated from bone marrow cells collected from the femurs of 8-week-old *fa/fa* rats and Wistar rats, followed by *in vitro* differentiation in high-glucose Dulbecco's modified Eagle's medium (DMEM; Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10 % heat-inactivated fetal bovine serum, 100U/ml penicillin/streptomycin, 2mM L-glutamine, and 10 ng/ml recombinant mouse macrophage colony-stimulating factor (M-CSF; Peprotech, Rocky Hill, NJ, USA). Cells were maintained in 5 % CO₂ at 37°C. On days 3 – 4, BMDMs were counted and seeded into 24-well plates (5 x 10⁵ cells/well). Cells were incubated overnight and then stimulated with 1 µg/ml of bacterial lipopolysaccharide (LPS, *E. Coli* O55:B5; Sigma-Aldrich, St. Louis, MO, USA). Cells and supernatants were harvested 24 hours after LPS stimulation for gene expression studies. IL-1α RT-qPCR and ELISA analyses were carried out as described above.

Statistical analysis

Data are presented as means ± standard errors (SEs). The analysis involved a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Student's t test for paired observations was used for the statistical comparison of BMDC IL-1α expression. Results were considered statistically significant at a p value <0.05.

Results

Array panel comparison of inflammatory cytokines and chemokines

We first evaluated plasma cytokine/chemokine profiles of WBN/Kob-*Lep^{fa}* (*fa/fa*) rats that developed diabetes, characterized by high plasma glucose levels at 9 weeks of age, using the cytokine/chemokine array panel (Fig. 1A). As shown in Fig. 1B, the array analysis revealed very low levels of cytokines and chemokines

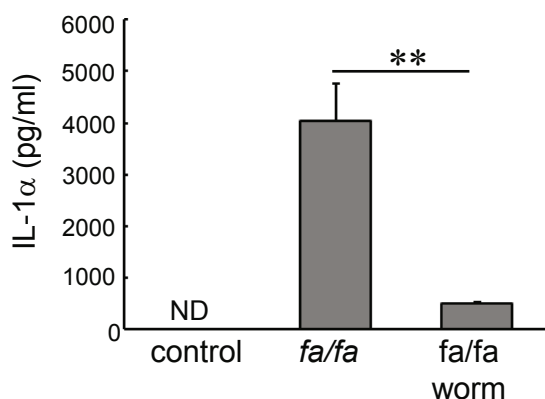


Fig. 2. Plasma IL-1α protein levels in control, *fa/fa* and *Syphacia* (*S.*) *muris*-infected *fa/fa* rats. Plasma IL-1α levels in Wistar rats (control), *fa/fa* rats (*fa/fa*), and *S. muris*-infected *fa/fa* rats (*fa/fa* worm) were measured by enzyme-linked immunosorbent assay. Data are presented as means ± standard errors (n = 4). **: p <0.01. ND = not detected.

in the plasma of control rats. In contrast, the plasma levels of most cytokines and chemokines were at least 2-fold higher in *fa/fa* rats than in control rats, although the signal at each spot was relatively weak (Fig. 1B). Among these elevated cytokines, we detected an extremely high level of IL-1α in plasma from *fa/fa* rats (Fig. 1B). The levels of IL-1α was higher than that of IL-1β, another IL-1 family cytokine that has been reported to associate with chronic inflammation in obesity (Fig. 1B).

fa/fa rats exhibit elevated plasma levels of IL-1α

We next examined the amount of IL-1α in plasma from control and *fa/fa* rats by ELISA. IL-1α was not detected in plasma from control

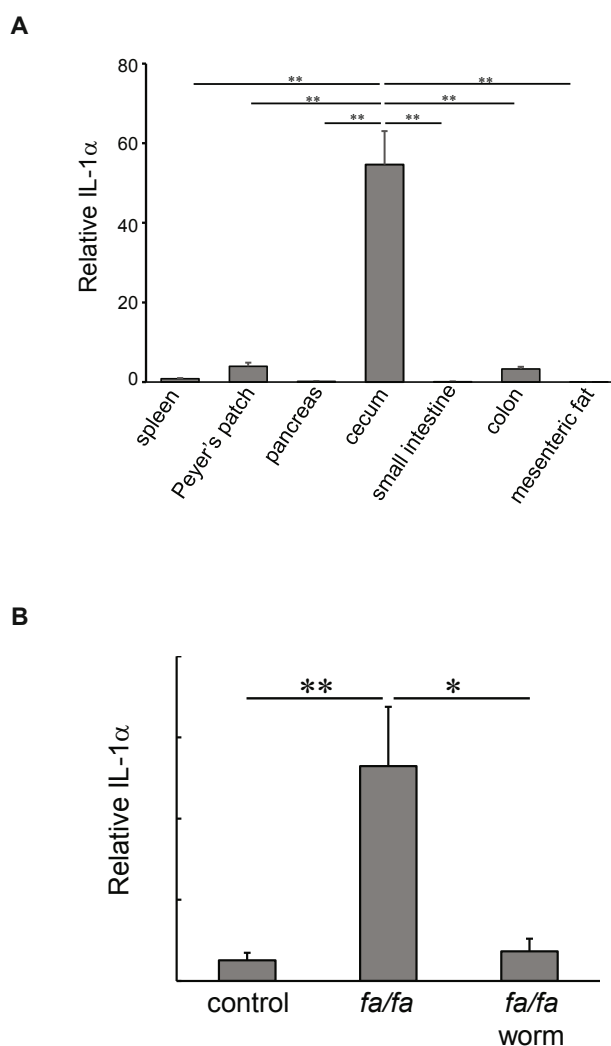


Fig. 3. Relative IL-1α mRNA levels in the ceca of control, *fa/fa* and *Syphacia* (*S.*) *muris*-infected *fa/fa* rats. (A) IL-1α mRNA expression levels among organs and tissues (spleen, Peyer's patch, pancreas, cecum, intestine, colon, mesenteric fat) were measured by real-time quantitative PCR. (B) IL-1α mRNA expression levels in the ceca of Wistar rats (control), *fa/fa* rats (*fa/fa*), and *S. muris*-infected *fa/fa* rats (*fa/fa* worm) were measured by real-time quantitative PCR. Data are expressed as means ± standard errors (n = 4). *: p <0.05, **: p <0.01.

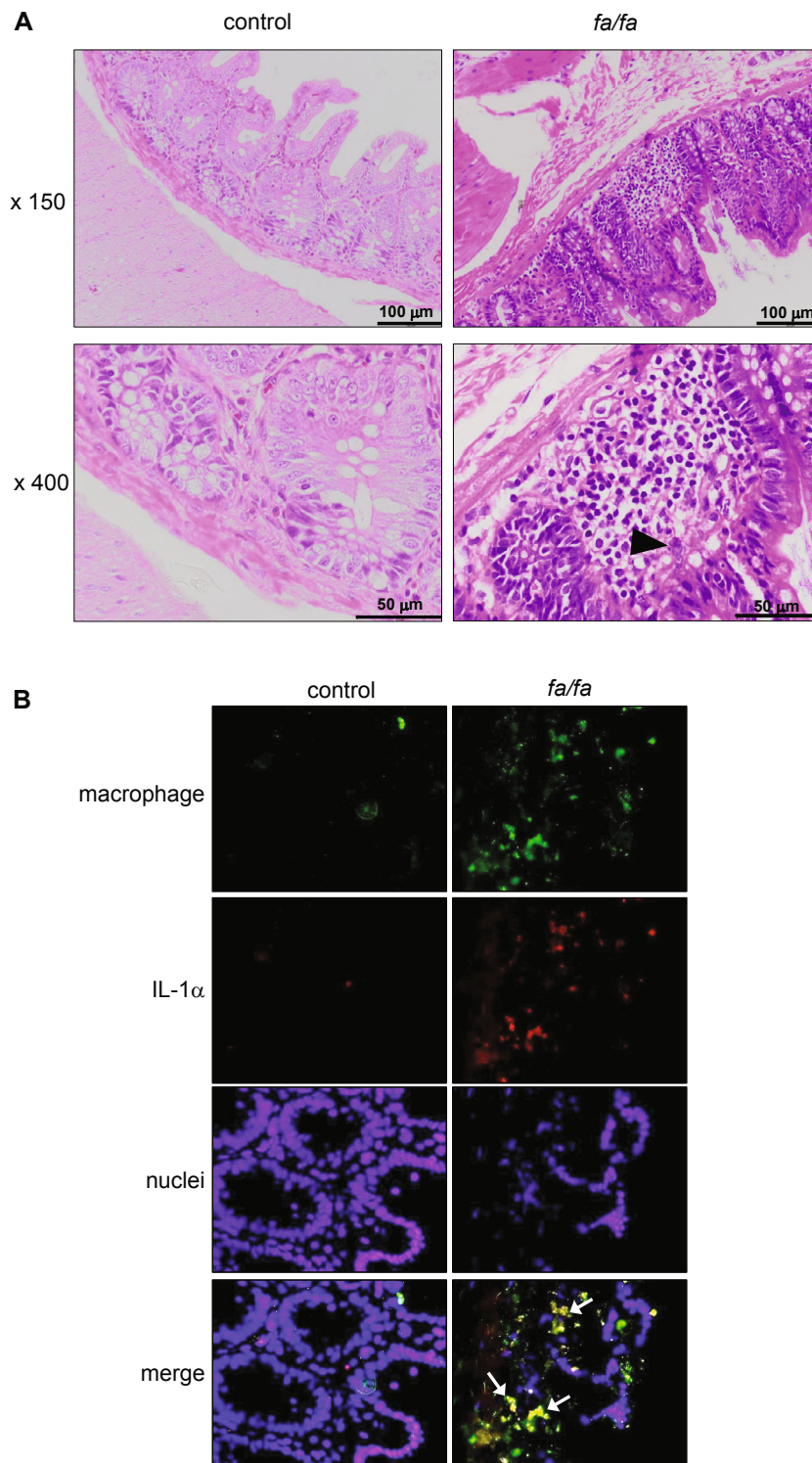


Fig. 4. Cecal inflammation with IL-1 α producing-macrophage infiltration observed in *fa/fa* rats.

(A) Representative hematoxylin and eosin-stained cecal sections from control and *fa/fa* rats. Low-powered (x150, top) and high-powered (x400, bottom) images of ceca of Wistar rats (control) and *fa/fa* rats (*fa/fa*). Mucosal infiltrates of lymphoid cells and macrophages (arrowhead) are visible in the ceca of *fa/fa* rats. (B) Representative immunohistochemical images of F4/80 and IL-1 α . Cecal sections of Wistar rats (control) or *fa/fa* rats (*fa/fa*) were stained with antibodies against macrophage marker F4/80 (green) and IL-1 α (red). Nuclei were stained with DAPI (blue). Images were captured at 150 \times magnification. Macrophages expressing IL-1 α (arrow) are visible in the ceca of *fa/fa* rats.

rats (Fig. 2). On the other hand, the amount of plasma IL-1 α in *fa/fa* rats was extremely elevated in comparison with control rats (Fig. 2); in other words, these results were similar to those obtained from the cytokine/chemokine array panel (Fig. 1B). These findings, taken together with the results in Fig. 1B, demonstrate that the pro-inflammatory cytokine IL-1 α is the main cytokine produced in *fa/fa* rats that have developed diabetes.

Ceca from *fa/fa* rats express high levels of IL-1 α mRNA

We used RT-qPCR to investigate IL-1 α expression in various organs of *fa/fa* rats in an attempt to identify the sources of the high levels of circulating IL-1 α . Total RNA was extracted from various organs or tissues (including spleen, Peyer's patch, pancreas, cecum, intestine, colon, mesenteric fat) with suspected involvement in the production of inflammatory cytokines associated with

the onset of T2DM. Among the organs and tissues analyzed in *fa/fa* rats, the highest levels of IL-1 α were found in the cecum (Fig. 3A). As shown in Fig. 3B, the IL-1 α mRNA expression levels in the ceca of control rats were very low, whereas those of *fa/fa* rats were significantly increased. IL-1 α expression was not detected in the spleen or pancreas in *fa/fa* rats (Fig. 3A). These results suggest that the cecum is one of the main sources of the high levels of plasma IL-1 α observed in *fa/fa* rats.

fa/fa rats exhibit cecal inflammation with macrophage infiltration

IL-1 α has recently been reported to associate with sterile inflammation (Freigang *et al.*, 2013). Because high levels of IL-1 α were expressed in the ceca of *fa/fa* rats with diabetes, a histological analysis of the ceca from *fa/fa* rats was conducted to examine the presence of inflammation. These histological findings are shown in Fig. 4A. Pathological cecal changes were not observed in control rats. In contrast, the ceca of *fa/fa* rats exhibited inflammatory cell infiltration into the lamina propria (Fig. 4A). Although focal inflammatory infiltrates in the cecal mucosa were mainly composed of lymphocytes, macrophage infiltration was also observed (Fig. 4A, arrowhead). We further subjected cecal sections from control or *fa/fa* rats with diabetes to an immunohistochemical analysis with antibodies against the macrophage marker F4/80 and against the C-terminal of IL-1 α . As shown in Fig. 4B, *fa/fa* rats contained increased numbers of F4/80-positive macrophages in the cecal mucosa, compared with control rats. Furthermore, IL-1 α protein-producing macrophages were detected in cecal sections of *fa/fa* rats, but not of control rats (arrows in Fig. 4B). These results suggest that macrophage infiltration and consequent enhanced IL-1 α production might induce cecal inflammation in *fa/fa* rats.

Increased IL-1 α production from *fa/fa* BMDMs upon LPS stimulation

Thus far, our results suggested that pro-inflammatory responses of macrophages are enhanced in *fa/fa* rats relative to control rats. To evaluate this further, we stimulated BMDMs from *fa/fa* or control rats with bacterial lipopolysaccharide (LPS), which induces pro-inflammatory cytokine production through toll-like receptor (TLR) 4 activation (Jin *et al.*, 2008; Kawai *et al.*, 2011). We observed no differences in macrophage differentiation rates, which were determined using a macrophage-specific antibody against F4/80 (Santa Cruz Biotechnology), between *fa/fa* or control rats. Very low levels of IL-1 α mRNA were detected in the absence of stimulation, and IL-1 α expression was induced by LPS stimulation in BMDCs from both genotypes (Fig. 6A). However, the IL-1 α mRNA expression levels in *fa/fa* BMDCs upon stimulation were significantly higher than those in control BMDCs (Fig. 6A). We also measured the amount of IL-1 α protein in culture supernatants; as shown in Fig. 6B, culture supernatants from LPS-stimulated *fa/fa* BMDCs contained much higher amounts of IL-1 α than did those from stimulated control BMDCs. These findings indicate that macrophages derived from *fa/fa* rats exhibit enhanced responsiveness to pro-inflammatory stimuli.

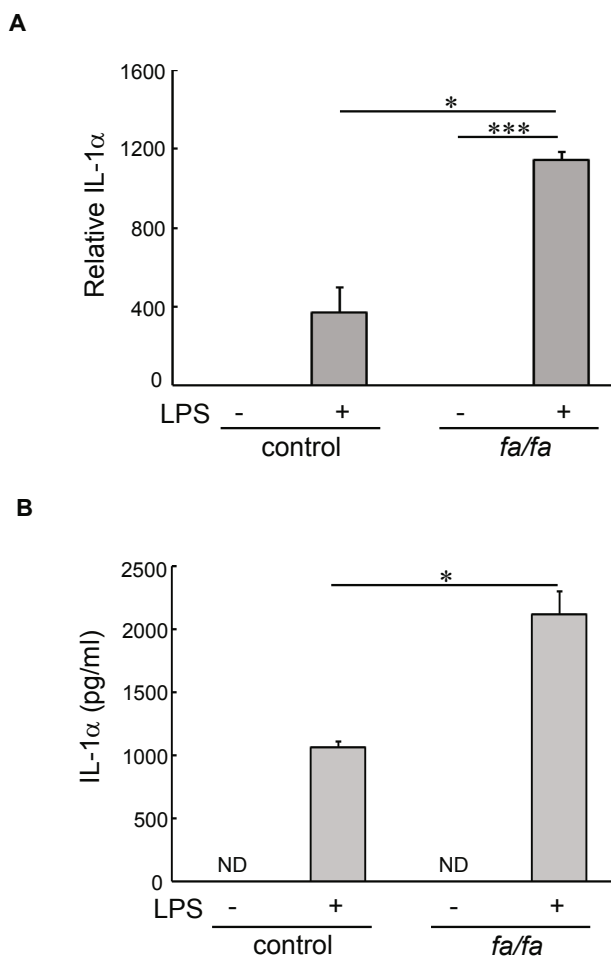


Fig. 5. Levels of IL-1 α produced by bone marrow-derived macrophages (BMDMs) from control and *fa/fa* rats. BMDMs from Wistar rats (control) or *fa/fa* rats (*fa/fa*) were unstimulated or stimulated with LPS (1 μ g/ml). (A) IL-1 α mRNA expression levels were determined by real-time quantitative PCR. (B) IL-1 α protein levels in supernatants were measured by enzyme-linked immunosorbent assay.

Data are expressed as means \pm standard errors (n = 3).

*: p < 0.05, **: p < 0.01, ***: p < 0.001.

fa/fa rats infected with *S. muris* decreases plasma cytokine/chemokine levels and especially prominent elevated level of IL-1 α in plasma and cecum

In a previous study, we demonstrated that infection with *S. muris* delayed the onset of T2DM in *fa/fa* rats, and suggested that this nematode infection might regulate the local production of cytokines/chemokines production that promote T2DM development (Taira *et al.*, 2015). To examine the effect of *S. muris* infection on cytokine/chemokine production in *fa/fa* rats, we first investigated changes in plasma cytokine/chemokine levels in *fa/fa* and *S. muris*-infected *fa/fa* rats. An array analysis of circulating cytokines/chemokines revealed that when compared with uninfected *fa/fa* rats, the plasma levels of most cytokines, including IL-1 β and chemokines were decreased in *S. muris*-infected *fa/fa* rats to the levels similar to those detected in control rats (Fig. 1B). Furthermore, we found that the levels of IL-1 α which were extremely high in *fa/fa* rats, were also dramatically reduced in *S. muris*-infected *fa/fa* rats (Fig. 1B). ELISA analysis also demonstrated a remarkable decrease in the plasma IL-1 α concentration in *fa/fa* rats infected with *S. muris*, compared with uninfected rats (Fig. 2). These results indicate that *S. muris* infection decreases the levels of circulating IL-1 α in *fa/fa* rats. We further analyzed IL-1 α mRNA levels by RT-qPCR to determine whether *S. muris* infection would affect IL-1 α expression in the ceca of *fa/fa* rats. As shown in Fig. 3, the relative expression level of IL-1 α mRNA in the ceca of *S. muris*-infected *fa/fa* rats was significantly reduced when compared with that observed in uninfected rats, and was similar to the level detected in control rats. These findings suggest that *S. muris* infection inhibits the expression of cecal IL-1 α in *fa/fa* rats with diabetes; in addition, this inhibition might delay the onset of T2DM by reducing cecal inflammation.

Discussion

Currently, chronic inflammation is well accepted as a critical factor in the onset and progression of T2DM (Donath *et al.*, 2011). Chronic inflammation is characterized by an increase in circulating inflammatory cytokines (Rodriguez-Hernandez *et al.*, 2013). In this study, we observed increased plasma levels of cytokines/chemokines in *fa/fa* rats with diabetes relative to those in age-matched control rats (Fig. 1B). Likely, this increase in circulating cytokines results from pancreatic inflammation, as this strain naturally exhibits pancreatitis at 7 – 9 weeks of age (Akimoto *et al.*, 2012). We detected high plasma levels of IL-1 α rather than IL-1 β which is known to associate with T2DM (Fig. 1B and Fig. 2). Although the circulating IL-1 β level was lower than the IL-1 α level in *fa/fa* rats, it remained almost 2.6-fold higher than the plasma IL-1 β level in control rats (Fig. 1B). This result suggests that IL-1 β might also be involved in the onset of diabetes in *fa/fa* rats.

Aberrant IL-1 α signaling is involved in a wide range of inflammatory diseases, including arthritis, atherosclerosis, and diabetes (Kamari *et al.*, 2011; Kamari *et al.*, 2007; Banerjee *et al.*, 2012; Rider *et al.*,

2013). However, when compared with IL-1 β , little is known about the contribution of IL-1 α to the development of T2DM. Recently, Freigang *et al.*, reported that IL-1 α was selectively produced and secreted from macrophages upon stimulation via fatty acids such as oleic acid, leading to atherogenesis via vascular inflammation (Freigang *et al.*, 2013; Spears *et al.*, 2013). In our present study, cecal IL-1 α expression was significantly higher in *fa/fa* rats with diabetes than in control rats (Fig. 3). Our data demonstrated elevated macrophage infiltration into the ceca of *fa/fa* rats, compared with control rats; in addition, the cecal macrophages in *fa/fa* rats expressed high levels of IL-1 α (Fig. 4). Furthermore, IL-1 α production in BMDMs derived from *fa/fa* rats in response to innate immune stimulation was significantly higher than in control BMDMs (Fig. 5). Recently, Jourdan *et al.*, reported that endocannabinoid-activated infiltrating macrophages led to islet β -cell loss in a rat model of T2DM (Jourdan *et al.*, 2013), suggesting that infiltrating macrophages and the consequent inflammatory cytokines play an important role in the onset of T2DM. Although the molecular mechanisms that control the increased production of IL-1 α from macrophages of *fa/fa* rats remains unclear, previous report noted that activation of the absent in melanoma 2 (AIM2) inflammasome led to the release of higher levels of IL-1 α but not IL-1 β via calpain activation (Sorrentino *et al.*, 2015). Likewise, our results suggest that in *fa/fa* rats, macrophages are activated by stimuli such as free fatty acids from adipocytes or islet amyloid polypeptides, thus inducing IL-1 α expression and secretion along with migration to the cecum.

We previously demonstrated that *S. muris* infection decreases plasma glucose level and delays the onset of T2DM in male *fa/fa* rats (Taira *et al.*, 2015). In the present study, *S. muris*-infected *fa/fa* rats exhibited decreased levels of circulating cytokines, including IL-1 α and IL-1 β and chemokines (Fig. 1B and Fig. 2). Furthermore, *S. muris*-infected *fa/fa* rats exhibited reduced cecal IL-1 α expression (Fig. 3B). These results suggest that *S. muris* could regulate the production of IL-1 α in the cecum, the site of parasitization for this nematode. Our previous study revealed that *S. muris* expresses mRNAs for Fc receptor signaling proteins that might control macrophage activation (Okamoto *et al.*, 2015). This report and our present study suggest that cecal macrophages are a candidate target for *S. muris*-mediated suppression of inflammatory reactions. We have not yet examined whether *S. muris* directly interacts with and regulates macrophage activation. In addition, it remains unclear whether a correlation exists between the cecal expression of IL-1 α and pancreatitis. Future studies intended to identify the involvement of IL-1 α or IL-1 α -producing macrophages in pancreatitis and the onset of T2DM and to elucidate the molecular mechanism underlying the *S. muris*-mediated inhibition of inflammatory cytokine production will be indispensable.

Conflict of interest

None of the authors have any conflict of interests.

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