

IL-33 is essential to prevent high fat diet-induced obesity in mice infected with an intestinal helminth

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Abstract

Intestinal helminthes induce immunosuppressive responses as well as type 2 immunity. Their suppressive properties are intended to regulate inflammatory diseases such as allergies and autoimmune diseases. This study evaluated whether helminthic infections suppress obesity, a chronic inflammatory state, using an intestinal nematode, *Heligmosomoides polygyrus* (Hp). Infection with Hp at the same time as feeding a high fat diet (HFD) prevented weight gain, dyslipidemia, and glucose intolerance observed in uninfected obese mice. Immunologically, Hp infection skewed M1 macrophages to M2 macrophages and induced type 2 innate lymphoid cells in adipose tissues. Expression of interleukin (IL)-33, a potent initiator of type 2 responses, was also increased in association with uncoupled protein 1 (UCP1). To further investigate the anti-obesity effects of IL-33 in mice infected with Hp, IL-33-deficient mice were fed the HFD and infected with Hp. These mutant mice rapidly gained weight compared with wildtype mice, indicating the anti-obesity effect of IL-33. In the absence of IL-33, the rapid increase in weight was not prevented, and type 2 responses and UCP1 expression were not observed even during Hp infection. These results suggested that the suppression of obesity by Hp is dependent on IL-33.

Introduction

Obesity raises the risk of metabolic syndromes that causes hypertension, diabetes mellitus, and myocardial infarction(1). Obesity results from accumulation of excessive energy as fat in adipocytes. Adipocytes themselves regulate energy metabolism to inhibit obesity by producing several bioactive molecules called adipokines. Some of them, such as adiponectin and leptin, are beneficial to inhibit obesity, whereas other inflammatory adipokines, such as tumor necrosis factor- α (TNF- α) and resistin, adversely affect developing obesity(2). In the obese state, inflammatory adipokines are predominantly produced, resulting in an increase in insulin resistance and induction of an influx of inflammatory cells into adipose tissues(3). Inflammatory responses caused by infiltrated cells in adipose tissues in turn promote developing obesity. Thus, obesity is suggested to be a chronic inflammatory disorder.

The incidence of obesity is increasing, especially in developed countries where infectious diseases have almost been eliminated. Because the “hygiene hypothesis” proposes that a sharp decrease in exposure to pathogens due to sanitization increases the risk of inflammatory diseases(4), the increase of obesity as a chronic inflammatory state

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might be explained by this theory. Among numerous infectious agents, helminthes are very unique to induce type 2 immune responses(5) and induce several immune regulatory cells. Together with the epidemiological inverse correlation between the incidence of inflammatory disorders and that of parasitic manifestation, helminthes including intestinal nematodes should contribute to the hygiene hypothesis. Several experimental models have demonstrated that intestinal helminthic infections suppress allergies and autoimmunity by modulating host immune responses(6). For instance, helminthic infections suppress inflammatory disorders through producing anti-inflammatory cytokines(7), shifting pathogenic Th1/17 to Th2 in autoimmune diseases(8), inducing regulatory T cells in allergy(9), and directly attenuating allergic Th2 responses(10). However, protective roles of helminthic infections against obesity are mostly unknown.

We recently reported that an intestinal nematode, *Heligmosomoides polygyrus* (Hp), suppresses weight gain in obese mice fed a high fat diet (HFD) by regulating hormones to consume calories in adipocytes(11). Su and colleagues demonstrated that a helminthic infection protects against HFD-induced obesity in an M2

macrophage-dependent manner(12), suggesting precise roles of type 2 immunity induced by helminthic infections in suppression of obesity. IL-33 is a potent initiator of type 2 immune responses including activation of type 2 innate lymphoid cells (ILC2) and M2 macrophages during helminthic infection (13). On the one hand, IL-33 contributes to the pathogenesis of type 2-mediated allergic responses (14), On the other hand it protects against several inflammatory conditions including obesity (15). To make the matter further complicated, helminthic infections induce IL-33 that activates protective type 2 immune responses(16), whilst they suppress IL-33 for their evasion from protective immunity(17). Thus, the involvement of IL-33 in protective roles of helminth-induced immunity against obesity is not well understood.

In this study, to evaluate the roles of IL-33 in protective effects of a helminthic infection on obesity, IL-33KO mice fed a HFD were infected with Hp. Infection of IL-33-sufficient mice with Hp suppressed weight gains, fat accumulation, dyslipidemia, and glucose intolerance. Immunologically, M1 macrophage-related inflammatory cytokines were suppressed, and type 2 responses, including M2 macrophages, ILC2, type 2 cytokines, and IL-33, were remarkably induced in adipose tissues, which was

associated with upregulation of UCP1. These type 2 responses were severely impaired and the suppressive effects on obesity were abolished in the absence of IL-33. Our results reveal that IL-33 is critical for anti-obesity effects exerted by intestinal helminthic infections.

Methods

Mice

Male 8-week-old C57BL/6J mice purchased from Japan SLC (Hamamatsu, Japan) were maintained under specific pathogen-free conditions and used for experiments at 10–12 weeks of age. Age and sex-matched IL-33^{-/-} mice, a generous gift from Dr. T. Yoshimoto (Hyogo College of Medicine)(18), were also used. For experimental feeding, a HFD containing 60% fat (HFD-60; Oriental Yeast Corporation, Tokyo, Japan) and control normal diet (AIN-93M, Oriental Yeast Corporation) were used. All animal experiments were reviewed and approved by the Committee for Ethics on Animal Experiments at the Graduate School of Gunma University (approval number 16-041) and were conducted under the control of the Guidelines for Animal Experiments at the Graduate School of Gunma University, and under the Law (No. 105) and Notification (No. 6) of the Japanese Government.

Helminthic infection

For all experiments, age matched 10–12-weeks-old mice were infected with 200 third

stage Hp larvae by oral gavage. The larvae were obtained from fecal cultures of eggs and stored at 4 °C. The course of infections was monitored by counting eggs in feces and adult worms in the small intestines. The pellets were feces were collected, measured the weight, and dissolved in PBS. Fecal solutions were mounted on slide glasses and observed under microscopy. Adult worms were collected from the small intestines from dissected mice.

Oral glucose tolerance test

Prior to the test, mice were fasted for 16 hours, followed by oral gavage of glucose at 2 g/kg body weight. Blood glucose levels were evaluated at 0, 15, 30, 60, 90, 120, 150, and 180 min post-injection using a labogluco (Funakoshi, Tokyo, Japan).

Metabolic parameters analysis

Serum levels of triglycerides and non-esterified fatty acids (NEFAs) were determined by using LabAssay (Wako, Tokyo, Japan), an enzymatic chromogenic quantification(19), according to the manufacturer's instructions.

qPCR Analysis

Total RNA was isolated from adipose tissue using an RNeasy mini kit (Qiagen, Hilden, Germany), and cDNA was obtained with RiverTraAce (TOYOBO, Osaka, Japan).

Resultant cDNA encoding genes of interest was quantified by real-time PCR using SYBR Green (TakaraBio, Shiga, Japan) in a Thermal Cycler Dice Real Time System (Takara) and normalized to mRNA encoding GAPDH in accordance with the manufacturer's protocol. The specific primer pairs were as follows: *Il4*,

5'-AGATGGATGTGCCAAACGTCCTCA-3' and

5'-AATATGCGAAGCACCTTGGAAGCC-3'; *Il5*,

5'-TGTTGACAAGCAATGAGACGATGA-3' and

5'-AATAGCATTTCCACAGTACCCCA-3'; *Il13*,

5'-TGAGGAGCTGAGCAACATCACACA-3' and 5'-

TGCGGTTACAGAGGCCATGCAATA-3'; *Il6*, 5'-CTTCCATCCAGTTGCCTTCTTG

-3' and 5'-AATTAAGCCTCCGACTTGTGAAG-3'; *Tnfa*,

5'-CCCGAGTGACAAGCCTGTAG-3' and 5'-GATGGCAGAGAGGAGGTTGAC-3';

Arg1, 5'-TTGGGTGGATGCTCACACTG-3' and
5'-GTACACGATGTCTTTGGCAGA-3'; *iNOS*,
5'-GTTCTCAGCCCAACAATACAAGA-3' and 5'-GTGGACGGGTCGATGTCAC-3';
Il33, 5'-CAATCAGGCGACGGTGTGGATGG-3' and
5'-TCCGGAGGCGAGACGTCACC-3'; *Ucp1*, 5'-ACTGCCACACCTCCAGTCATT-3'
and 5'-CTTTGCCTCACTCAGGATTGG-3'; *Gapdh*,
5'-TGTGTCCGTCGTGGATCTGA-3' and 5'-TTGCTGTTGAAGTCGCAGGAG-3'.

Flow cytometric analysis

Macrophages in adipose tissue were collected as described previously(20). The adipose tissue was excised from the mouse and minced with scissors. The tissue was digested for 30 min in a solution containing 2 mg/mL collagenase type II at 37 °C. After digestion, the tissue was filtered through a nylon mesh (100 µm) and washed with RPMI1640 (Wako, Osaka, Japan). The filtered cells were separated to obtained adipocytes and mononuclear cells. Finally, the each cell type was washed, centrifuged, and re-suspended in FACS buffer. The purified mononuclear cells stained with

fluorescent-label antibodies were analyzed by a FACS Verse (Becton Dickinson, New jersey, USA). M1 macrophages were identified as F4/80⁺ CD11c⁺CD206⁻ cells, and M2 macrophages were identified as F4/80⁺CD11c⁻ CD206⁺ cells. ILC2 were identified as Lineage⁻CD45⁺CD127⁺KLRG-1⁺ST2⁺ cells. Data analysis was performed using Flowjo software (tree Star, Ashland, OR, USA).

Statistical analysis

Statistical analysis was performed using Prism software (v.6.0e) and R (v.3.2.4).

Statistical differences were evaluated using unpaired Student's t test and two-way

ANOVA, followed by Tukey's post-hoc test. P-values of <0.05 were considered as significant.

Results

Parasitic infection suppresses weight gain by a high fat diet

First, to determine whether parasitic infection suppresses obesity, mice infected with an intestinal nematode, *Heligmosomoides polygyrus* (Hp), while simultaneously feed a high fat diet (HFD) or control normal diet (ND) (Fig. 1A). Mice fed with ND gained weight slowly as they grew, and infection with Hp did not affect their body weight.

However, mice fed with HFD showed a marked increase in body weight up to 30% greater than that of control mice (Fig. 1B). In addition, a large amount of visceral and subcutaneous white adipose tissues (WAT) had accumulated in these mice (Fig 1C, D).

Infection with Hp resulted in a significant reduction in body weight from as early as day 14 after infection (Fig. 1B). Consistent with the suppression of weight gain, accumulation of WAT was also significantly reduced by Hp infection (Fig. 1C, D).

Parasitic infection also normalized blood triglycerides and non-esterified free fatty acids (NEFAs) that had been increased in mice fed the HFD (Fig. 1E, F). Feeding on the HFD for 28 days did not induce hyperglycemia, but caused less tolerance to hyperglycemia as measured by the oral glucose tolerance test (OGTT). In the OGTT, hyperglycemia

persisted in HFD-fed mice, which was rapidly decreased upon Hp infection (Fig. 1G).

HFD-fed mice harbored adult worms and produced eggs similarly to those fed with NFD (Fig. 1H, I). Based on these results, infection with Hp suppresses weight gain in HFD-fed mice.

Parasite infection suppresses M1-related inflammation in obese mice

Because it is believed that obesity is related to chronic inflammatory conditions, we analyzed inflammation in WAT of HFD-fed mice. When mice were fed with HFD, transcription of genes encoding inflammatory cytokines IL-6 and TNF- α was enhanced remarkably (Fig. 2A). In addition, more macrophages, particularly M1 macrophages, were recovered from WAT (Fig. 2B). Predominance of M1 macrophages was confirmed by increased transcripts of their effector molecule, inducible nitric oxide synthase (Fig. 2C). These results revealed that HFD-induced obesity was accompanied by inflammation. Infection of HFD-fed mice with Hp reduced expression of IL-6 and TNF- α (Fig. 2A), infiltration of M1 macrophages into WAT, and expression of iNOS (Fig. 2B). Moreover, infection of Hp induced M2 macrophages and their effector

molecule, arginase 1 (Fig. 2B, C). These results demonstrate that Hp infection suppresses inflammatory responses in HFD-induced obese mice in association with increase of anti-inflammatory M2 macrophages and decrease of inflammatory M1 macrophages.

Parasitic infection induces type 2 immune responses in adipose tissue

We next analyzed type 2 immune responses in Hp-infected mice, because intestinal nematodes are known to widely induce type 2 responses. After infection with Hp, expression of typical type 2 cytokines IL-4, IL-5, and IL-13 was elevated regardless of the type of diet, and the degree in increase was greater in HFD-fed mice compared with control mice (Fig. 3A). Furthermore, ILC2(Supplementary Fig.1), a main producer of these cytokines, was increased, especially in HFD-fed mice (Fig. 3B). Because IL-33 plays an important role in the induction of ILC2, expression of IL-33 was analyzed in WAT. In the absence of Hp infection, almost no IL-33 was detected in WAT. In contrast, Hp infection significantly increased mRNA encoding IL-33, which was more obvious in HFD-fed mice (Fig. 3C). As Hp induces production of IL-1 β that suppresses IL-33

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production(17), it is possible that remarkable increase of IL-33 in HFD-fed mice infected with Hp was attributed to less amount of suppressive IL-1 β . To test this possibility, mRNA encoding IL-1 β was measured in the adipose tissues. However, this was unlikely because more IL-1 β was detected in HFD-fed mice after infection with Hp (Fig. 3D). Thus, a wide variety of type 2 responses were evoked in WAT after infection with Hp. To relate the induction of type 2 responses to suppression of weight gain in Hp-infected obese mice, we next analyzed expression of uncoupling protein 1 (UCP1), because an increase of ILC2 is known to induce expression of this mitochondrial protein that metabolizes fat in adipocytes(21). The expression of UCP1 was enhanced after Hp infection, especially in HFD-fed mice, and was closely correlated with the amount of ILC2 (Fig. 3E).

Weight loss in parasite-infected mice is dependent on IL-33

ILC2 and its potent inducer IL-33 have been thought to limit obesity. Indeed, IL-33 and ILC2 were increased in adipose tissue of HFD-fed mice infected Hp. To determine whether IL-33 is involved in obesity control of these mice, we fed IL-33 KO mice with

HFD. As reported previously(22), feeding IL-33 KO mice with HFD exacerbated obesity and dyslipidemia compared with WT mice. Although infection of HFD-fed WT mice with Hp improved these measurements as well glucose tolerance, that of IL-33 KO mice did not (Fig. 4A–D). Immunological analyses revealed that the attenuation of M1-related inflammation observed in WT mice infected with Hp did not occur in the absence of IL-33 (Fig. 4E, F). In contrast, augmentation of type 2 immune responses, such as production of IL-4, IL-5, and IL-13 and induction of ILC2 in WT mice infected with Hp, was very limited in IL-33 KO mice (Fig. 4G, H). Finally, induction of UCP1 presumably responsible for suppressive effects of Hp infection on weight gain was highly impaired in the absence of IL-33 (Fig. 4I). Thus, infection with Hp did not suppress weight gain of IL-33 KO mice, which was not due to refractoriness of these mutant mice to the infection. IL-33 KO mice appeared to be rather more susceptible as judged by significantly more eggs of Hp produced in these mice (Fig. 4J). These results demonstrate that IL-33 is indispensable for suppression of obesity caused by Hp infection.

Discussion

Here, we describe immunological aspects of anti-obesity properties observed in mice infected with Hp. Simultaneous infection with Hp and HFD feeding significantly suppressed weight gain. It induced M2 macrophages instead of inflammatory M1 macrophages and ILC2 in adipose tissues. Furthermore, UCP1 expression was enhanced in mice infected with Hp, which might be responsible for suppression of weight gain due to calorie expenditure in adipocytes. Suppressing effects on obesity and immunological alterations in Hp-infected mice were dependent on IL-33. This is the first report demonstrating a crucial role of IL-33 in helminth-induced prevention of obesity, although infection with Hp is well documented to induce type 2 immunity in an IL-33-dependent manner and prevent obesity. Preventive responses such as type 2 cytokines production induced by Hp infection were more obvious in HFD-fed mice, and HFD alone seemed to enhance type 2 responses. It is postulated that the enhancement of type 2 responses in HFD-fed mice might be a compensatory response to suppress obesity and Hp infection might boost it.

IL-33 is reported to limit obesity by inducing ILC2 in adipose tissues(21). Then,

ILC2 produces Met-enkephalin that induces adipocytes to increase UCP1 expression.

Although we did not address whether Hp infection increases Met-enkephalin,

IL-33-dependent ILC2 accumulation in adipose tissues might play a major role in

Hp-induced anti-obesity effects. However, mechanisms other than induction of ILC2

may contribute to limiting weight gain. Suppression of weight gain in IL-33KO mice

infected with Hp was almost completely impaired (Fig. 4A), although these mice still

had increased numbers of ILC2 (Fig. 4h) presumably via other cytokines such as IL-25.

For example, IL-33 also regulates adipose tissue-resident CD4⁺ regulatory T cells that

prevent obesity-related inflammation(23). Because Hp infection is known to induce

CD4⁺ Tregs, an increase of these cells in adipose tissues may be involved in suppression

of weight gain. However, IL-33 is known to activate the sympathetic nervous

system(24) whose product, norepinephrine, induces UCP1 expression in adipocytes(25).

We previously reported that Hp infection suppresses obesity by increasing

norepinephrine in both adipose tissues and serum(11). Thus, IL-33-mediated

sympathetic nerve stimulation may suppress weight gain.

Intestinal helminthes including Hp evoke profound type 2 responses initiated by

IL-33 production from intestinal epithelial cells damaged by contact with migrated worms(26). Induction of type 2 immunity occurs not only in gut-associated lymphoid tissues, but also systemic lymphoid organs, suggesting that IL-33 is produced by several cells in various tissues. Indeed, our results indicated that Hp infection increased mRNA encoding IL-33 in adipose tissues, and one likely cellular source is adipocytes reported to produce IL-33(27). Together with enriched localization of ILC2 that highly respond to IL-33 in adipose tissues, this cytokine is presumably produced as a benign adipokine that regulates weight gain. Infection with Hp activates adipocytes to produce IL-33, although how the intestinal nematode affects adipose tissues is still unclear. Several cell types other than adipocytes including fibroblasts(28), endothelial cells(29), and macrophages(30) have been reported to produce IL-33. We did not exactly identify cellular source of IL-33 in this study, and those cells may be involved.

One possible mechanism underlying crosstalk between the intestinal nematode infection and adipocytes is modulation of intestinal microbiota, considering the habitat of Hp and effects of microbiota on metabolism and immune responses(31). In fact, we have reported that Hp influences intestinal microbiota to produce NE, resulting in

improvement of established obesity(11). It would be interesting to further evaluate the involvement of microbiota in Hp-mediated suppression of weight gain. Such insights might provide novel probiotic approaches to prevent obesity.

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Abbreviations used in the manuscript

Hp; *Heligmosomoides polygyrus*, HFD; high fat diet, ILC2; type 2 innate lymphoid cell,

UCP1; uncoupled protein 1, NEFA; non-esterified fatty acid, WAT; white adipose tissue

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A.O., T.I. and K.S. contributed to extensive discussions and critical comments. H.H.

supervised the project, designed the experiments, and wrote the paper.

Conflict of interest

The authors declare that there is no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding

author upon responsible request.

Figure legends

Figure 1. Infection with *Heligmosomoides polygyrus* (Hp) suppresses weight gain in HFD-fed mice. **(A)** Time scheduled of the experimental procedures. **(B)** Body weight of ND- and HFD-fed mice in the presence or absence of Hp was monitored periodically. **(C)** Macroscopic appearance of visceral (arrow) and epididymal (arrowhead) adipose tissues of the indicated mice. **(D)** Weight of epididymal adipose tissue, **(E)** blood concentration of triglycerides, **(F)** and non-esterified fatty acids (NEFAs) in the indicated groups. **(G)** Blood glucose concentration in the indicated mice was monitored after oral glucose intake. All measurements were performed using mice at 28 days after infection. **(H)** Number of Hp eggs per gram of feces. **(I)** Numbers of adult worms in the small intestines in the indicated mice at 4 and 12 weeks after infection. All values are presented as the mean $\pm$ standard deviation (SD) of from 15 mice in three individual experiments using 5 mice except for I from one experiment using 6 mice. Symbols in scatter graphs with bars represent data from an individual animal. Asterisks denote statistical significance at *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 by the Student's *t*-test or two-way ANOVA.

Figure 2. Infection with Hp attenuates M1-related inflammatory responses in HFD-induced obese mice. **(A)** mRNAs encoding IL-6 and TNF- α in total RNA extracted from epididymal adipose tissue of the indicated mice were quantified by real-time RT-PCR. The expression level relative to GAPDH mRNA is shown. **(B)** Numbers of F4/80⁺ total macrophages, F4/80⁺CD11c⁺CD206⁻ M1, and F4/80⁺CD11c⁺CD206⁺ M2 macrophages among mononuclear cells isolated from epididymal adipose tissue was calculated by flow cytometric analyses. The ratio of M1 to M2 macrophages was also calculated. **(C)** mRNAs encoding inducible nitric oxide synthase (iNOS) and arginase 1 (Arg 1) in total RNA extracted from epididymal adipose tissue of the indicated mice was quantified as described in **(A)**. All measurements were performed using mice at 28 days after infection. All values in are presented as the mean $\pm$ SD of 15 mice in three repeated experiments except for B from two experiments using 4 mice each. Symbols in scatter graphs with bars represent data from an individual animal. Asterisks denote statistical significance at *P < 0.05, **P<0.01, ***P<0.001, and ****P<0.0001 by the Student's *t*-test or two-way ANOVA.

Figure 3. Type 2 responses and expression of UCP1 are augmented in HFD-fed mice infected with Hp. mRNAs encoding type 2 cytokines IL-4, IL-5, and IL-13 (**A**), ILC2 inducer IL-33 (**C**), IL-1 β (**D**) and mitochondrial fat burning protein UCP-1 (**E**) were quantified by real-time RT-PCR. The expression level relative to GAPDH mRNA is shown. (**B**) The number of Lin⁻CD45⁺CD127⁺KLRG-1⁺ST2⁺ ILC2 in mononuclear cells isolated from epididymal adipose tissue was calculated by flow cytometric analyses. All measurements were performed using mice at 28 days after infection. All values are presented as the mean $\pm$ SD of 15 mice in three repeated experiments. Symbols in scatter graphs with bars represent data from an individual animal. Asterisks denote statistical significance at *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 by the Student's *t*-test or two-way ANOVA.

Figure 4. IL-33 is essential for anti-obesity effects of Hp infection. IL-33 KO mice fed with HFD were infected with Hp, and then (**A**) body weight, (**B**) blood triglycerides, (**C**) blood NEFAs, and (**D**) glucose homeostasis after oral glucose intake were evaluated

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as described in Fig. 1. (E) Inflammatory responses, (F) macrophage populations as described in Fig. 2, (G) type 2 cytokines, (H) ILC2, and (I) UCP1 expression in adipose tissue as described in Fig. 3 were also evaluated in HFD-fed IL-33 KO in the presence or absence of Hp infection. (J) The number of Hp eggs of feces was monitored weekly after infection of WT and IL-33 KO mice fed an HFD. All values are presented as the mean $\pm$ SD of 15 mice in three repeated individual experiments. Symbols in scatter graphs with bars represent data from an individual animal. Asterisks denote statistical significance at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ by the Student's *t*-test or two-way ANOVA.

Supplemental Figure 1. Gating strategies used in flow cytometry analyses. (A) ICL2 cells were determined as Lineage⁻CD45⁺CD127⁺KLRG1⁺ST2⁺ cells in FSC/SSC-gated lymphoid cells. (B) M1 and M2 macrophages were determined as CD11c⁺CD206⁻ and CD11c⁺CD206⁺ cells, respectively, after gating lymphoid F4/80⁺ cells.

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Fig. 1.

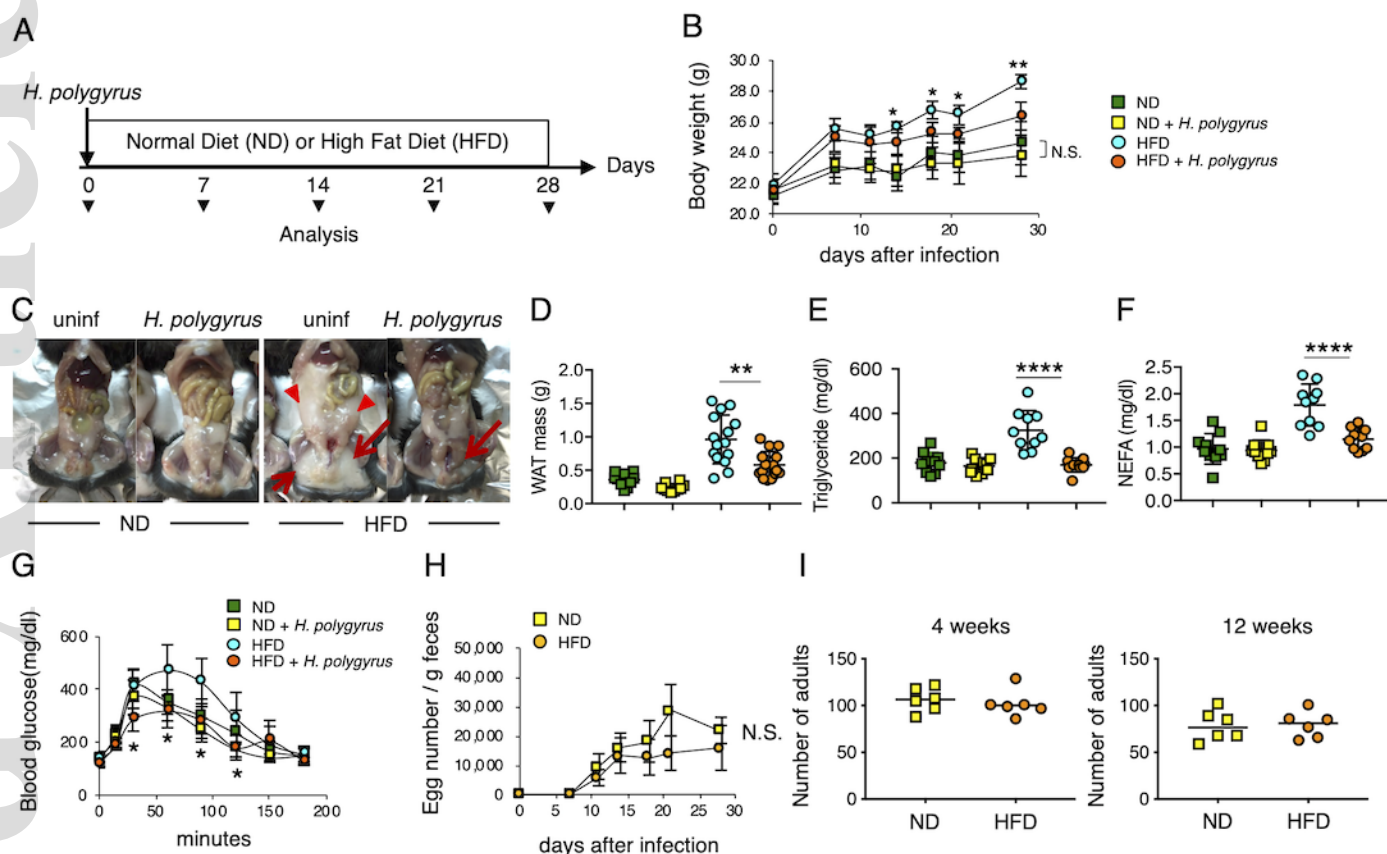


Fig. 1.

Infection with *Heligmosomoides polygyrus* (Hp) suppresses weight gain in HFD-fed mice. (A) Time scheduled of the experimental procedures. (B) Body weight of ND- and HFD-fed mice in the presence or absence of Hp was monitored periodically. (C) Macroscopic appearance of visceral (arrow) and epididymal (arrowhead) adipose tissues of the indicated mice. (D) Weight of epididymal adipose tissue, (E) blood concentration of triglycerides, (F) and non-esterified fatty acids (NEFAs) in the indicated groups. (G) Blood glucose concentration in the indicated mice was monitored after oral glucose intake. All measurements were performed using mice at 28 days after infection. (H) Number of Hp eggs per gram of feces. (I) Numbers of adult worms in the small intestines in the indicated mice at 4 and 12 weeks after infection. All values are presented as the mean $\pm$ standard deviation (SD) of from 15 mice in three individual experiments using 5 mice except for I from one experiment using 6 mice. Symbols in scatter graphs with bars represent data from an individual animal. Asterisks denote statistical significance at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ by the Student's *t*-test or two-way ANOVA.

Fig. 2.

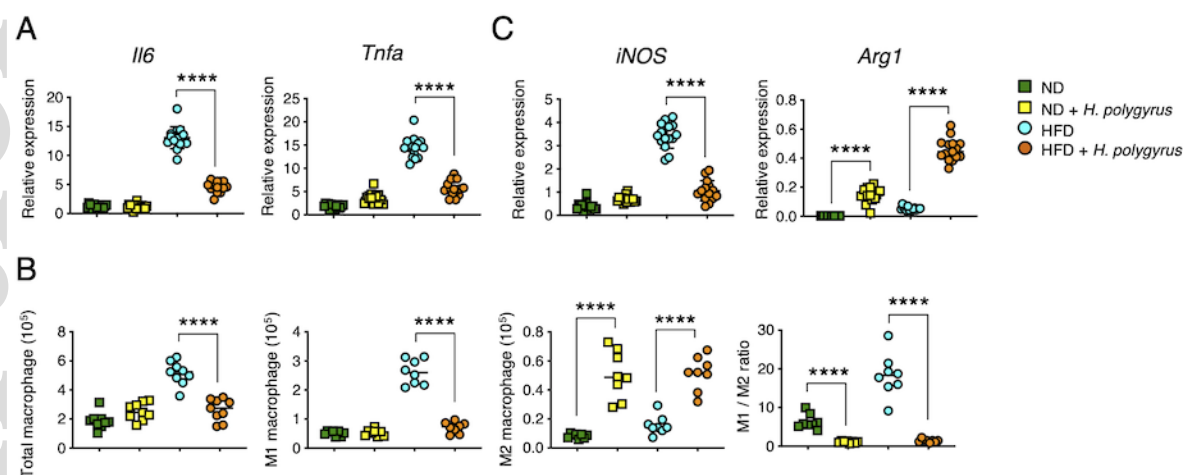


Fig. 2.

Infection with *Hp* attenuates M1-related inflammatory responses in HFD-induced obese mice. (A) mRNAs encoding IL-6 and TNF- α in total RNA extracted from epididymal adipose tissue of the indicated mice were quantified by real-time RT-PCR. The expression level relative to GAPDH mRNA is shown. (B) Numbers of F4/80⁺ total macrophages, F4/80⁺CD11c⁺CD206⁻ M1, and F4/80⁺CD11c⁺CD206⁺ M2 macrophages among mononuclear cells isolated from epididymal adipose tissue was calculated by flow cytometric analyses. The ratio of M1 to M2 macrophages was also calculated. (C) mRNAs encoding inducible nitric oxide synthase (iNOS) and arginase 1 (Arg 1) in total RNA extracted from epididymal adipose tissue of the indicated mice was quantified as described in (A). All measurements were performed using mice at 28 days after infection. All values in are presented as the mean $\pm$ SD of 15 mice in three repeated experiments except for B from two experiments using 4 mice each. Symbols in scatter graphs with bars represent data from an individual animal. Asterisks denote statistical significance at *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 by the Student's *t*-test or two-way ANOVA.

Fig. 3.

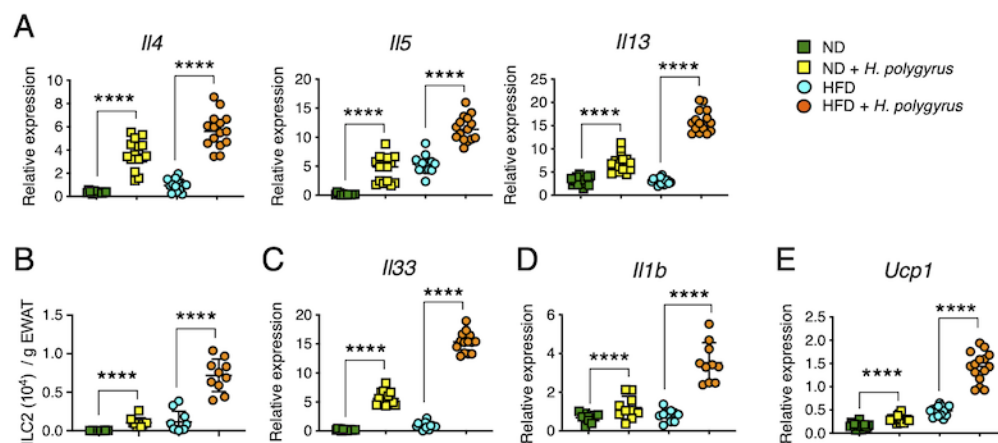


Fig. 3.

Type 2 responses and expression of UCP1 are augmented in HFD-fed mice infected with *Hp*. mRNAs encoding type 2 cytokines IL-4, IL-5, and IL-1 β (A), ILC2 inducer IL-33 (C), IL-1 β (D) and mitochondrial fat burning protein UCP-1 (E) were quantified by real-time RT-PCR. The expression level relative to GAPDH mRNA is shown. (B) The number of Lin⁻ CD45⁺CD127⁺KLRG-1⁺ST2⁺ ILC2 in mononuclear cells isolated from epididymal adipose tissue was calculated by flow cytometric analyses. All measurements were performed using mice at 28 days after infection. All values are presented as the mean $\pm$ SD of 15 mice in three repeated experiments. Symbols in scatter graphs with bars represent data from an individual animal. Asterisks denote statistical significance at *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 by the Student's *t*-test or two-way ANOVA.

Fig. 4.

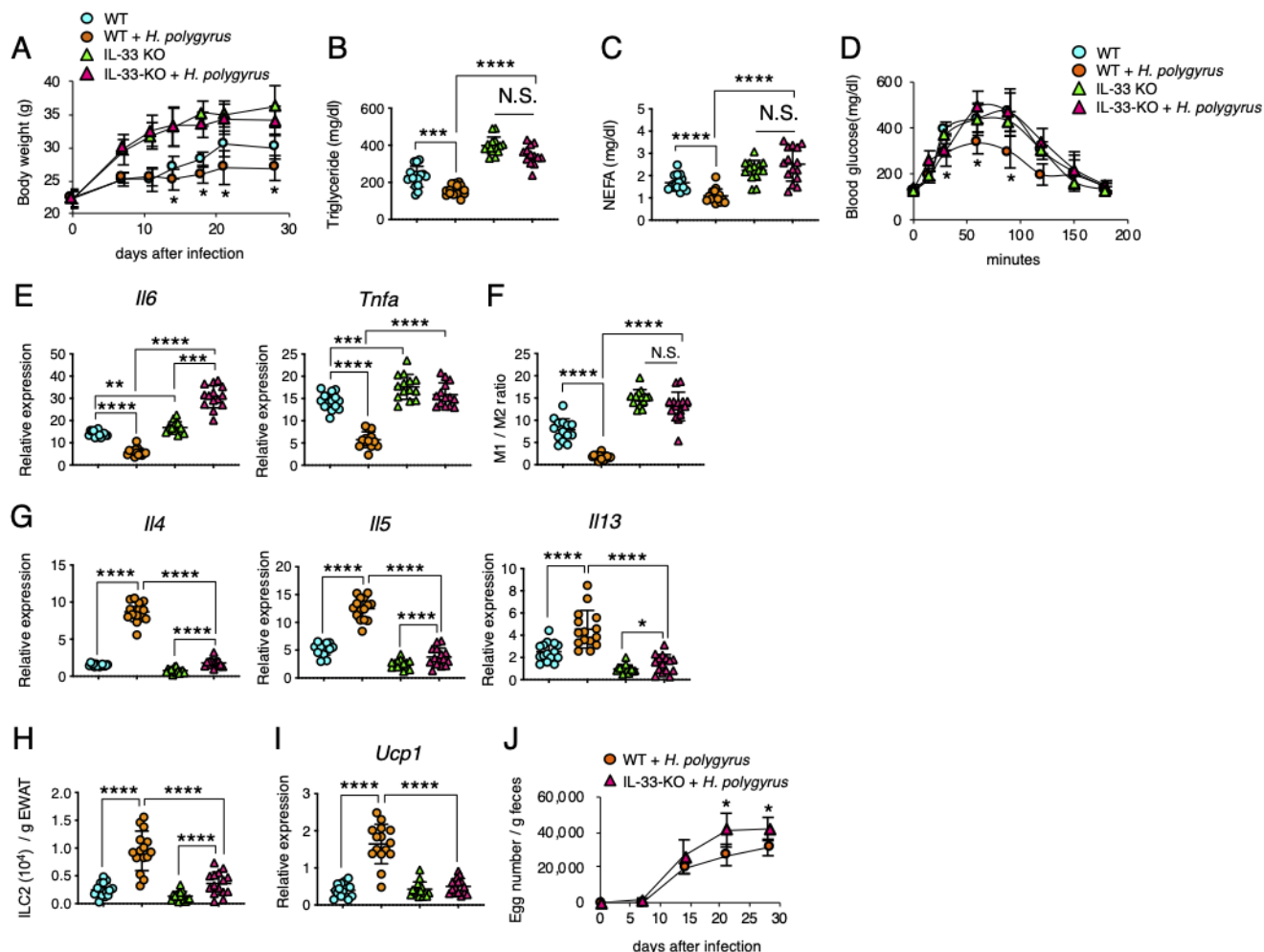
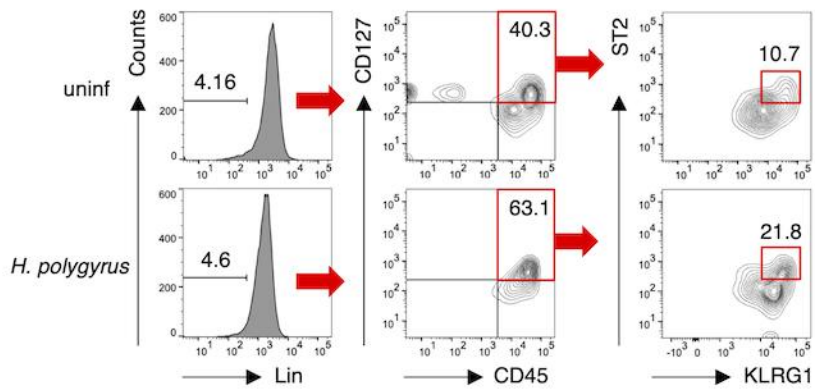


Fig. 4.

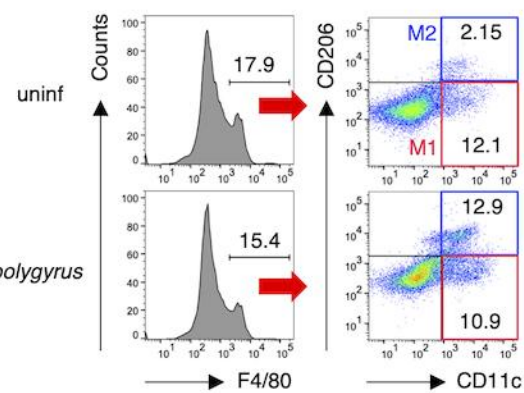
IL-33 is essential for anti-obesity effects of Hp infection. IL-33 KO mice fed with HFD were infected with Hp, and then (A) body weight, (B) blood triglycerides, (C) blood NEFAs, and (D) glucose homeostasis after oral glucose intake were evaluated as described in Fig. 1. (E) Inflammatory responses, (F) macrophage populations as described in Fig. 2, (G) type 2 cytokines, (H) ILC2, and (I) UCP1 expression in adipose tissue as described in Fig. 3 were also evaluated in HFD-fed IL-33 KO in the presence or absence of Hp infection. (J) The number of Hp eggs of feces was monitored weekly after infection of WT and IL-33 KO mice fed an HFD. All values are presented as the mean $\pm$ SD of 15 mice in three repeated individual experiments. Symbols in scatter graphs with bars represent data from an individual animal. Asterisks denote statistical significance at *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 by the Student's *t*-test or two-way ANOVA.

Supplementary figure 1

A



B



Supplementary figure 1

Gating strategies used in flow cytometry analyses. (A) ICL2 cells were determined as Lineage⁻CD45⁺CD127⁺KLRG1⁺ST2⁺ cells in FSC/SSC-gated lymphoid cells. (B) M1 and M2 macrophages were determined as CD11c⁺CD206⁻ and CD11c⁺CD206⁺ cells, respectively, after gating lymphoid F4/80⁺ cells.