ARTICLE

High-fat diet-induced resistance to helminth infection via alternative induction of type 2 immunity

Evelyn Funjika [1,2,3], Stefano A.P. Colombo [1,2], Kelly S. Hayes [1,2], Mary J. Tozer [4], Katrina A. Tyrrell [4], Shanshan Cai [4], Aduragbemi A. Faniyi [4], Rebecca K. Shears [1,2], Megan Dooley [4], Yasmine Alshammari [5], Wafaa Alhazmi [6], Mushref Assas [6], Abdullah Almilaibary [7], Lucy H. Jackson-Jones [4], David J. Thornton [1,2,✉], John J. Worthington [4,✉] and Richard K. Grencis [1,2,✉]

Gastrointestinal nematode infections cause morbidity and socioeconomic loss in the most deprived communities. The shift in the context of obesity has led to spatial overlap with endemic gastrointestinal nematode regions resulting in the emergence of a novel comorbidity. Despite this, the impact of a high-fat diet (HFD) on immune-regulated protection against gastrointestinal infections remains largely unknown. We employed the murine model of nematode infection, *Trichuris muris*, to investigate the effect of an HFD on the immune response against chronic infection. Surprisingly, diet-induced obesity drove parasite expulsion in both single and repeated trickle low doses of *T. muris* eggs. Mechanistically, an HFD increased the expression of the ST2 receptor on CD4+ T cells, priming an enhanced type 2 helper T (Th2) cell cytokine production following interleukin (IL)-33 stimulation *ex vivo*. Despite IL-33−/− mice demonstrating that IL-33 is not critical for host protective immunity to *T. muris* under a conventional diet, HFD-fed T-cell deplete mice adoptively transferred with ST2−/− CD4 T cells were unable to expel a *T. muris* infection unlike those transferred with ST2-sufficient cells. Collectively, this study demonstrates that an HFD primes CD4+ T cells to utilize the IL-33-ST2 axis in a novel induction of type 2 immunity, providing insights into the emerging comorbidities of obesity and nematode infection.

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INTRODUCTION

Trichuris trichiura is a major soil-transmitted helminth affecting over 400 million people worldwide causing trichuriasis[1]. Infection is rarely fatal, with associated symptoms including diarrhea, abdominal pain, intestinal bleeding, anemia, as well as nutritional deficiencies[1,2]. Further cross-sectional studies have linked malnutrition with nematode infection as a cause of stunted growth, with specific nutritional deficiencies in pediatric hosts including vitamin A, iron, and zinc[3].

Recently, low- to middle-income countries have also seen an increase in non-communicable diseases such as obesity[4,5]. This has resulted in the adoption of new diets containing highly processed foods that have high levels of refined sugars and fat[4]. Therefore, low- to middle-income countries now have the emerging double burden of soil-transmitted helminth infections and obesity-inducing diets.

The naturally occurring infection of *Trichuris muris* in mice models immune responses to *T. trichiura*, as the two species have similarities in morphology and genome, occupying the same intestinal niche and sharing antigenic cross-reactivity[6,7]. Multiple factors play a role in determining the immune response to *T. muris*, including infection dose[8], but the balance of the type 1 and 2 T helper (Th1/Th2) $CD4^+$ T-cell immune response is crucial in determining the outcome of infection[9]. Host nutrition also affects intestinal immunity with murine models of high-fat diet (HFD)-induced obesity associated with increased permeability of the intestinal barrier[10], resulting in low-grade systemic inflammation in the host[11].

In this study, we used *T. muris* infection in C57BL/6 mice to investigate the effect of HFD on the immune response to helminth infection. Our results show that although interleukin (IL)-33 is not critical for host protective immunity to *T. muris* in mice fed with a conventional diet, an HFD increased the action of IL-33 through the upregulation of CD4 T-cell IL-33 receptor ST2, enhancing classical Th2 immune responses and parasite expulsion. Collectively, this brings new insights into the emerging comorbidities of human obesity and nematode infection and may inform potential therapeutic approaches.

RESULTS

HFD-induced obesity results in the expulsion of a *T. muris* low-dose infection

To examine the relationship between the comorbidities of an HFD and chronic helminth infection, we utilized the *T. muris*

[1]The Lydia Becker Institute of Immunology and Inflammation, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK. [2]Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, Manchester, UK. [3]School of Natural Sciences, Department of Chemistry, The University of Zambia, Lusaka, Zambia. [4]Biomedical and Life Sciences, Faculty of Health and Medicine, University of Lancaster, Lancaster, UK. [5]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Kuwait University, Kuwait City, Kuwait. [6]Faculty of Applied Medical Sciences, Department of Medical laboratory technology, King AbdulAziz University, Jeddah, Saudi Arabia. [7]Faculty of Medicine, Albaha University, Albaha, Saudi Arabia. ✉email: dave.thornton@manchester.ac.uk (D.J. Thornton), j.j.worthington@lancaster.ac.uk (J.J. Worthington), richard.grencis@manchester.ac.uk (R.K. Grencis)

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system[9]. Wild-type (WT) C57BL/6 mice received an HFD or the corresponding control diet (CD) before the infection with either a single low dose of eggs, which normally results in chronic infection, or a trickle low-dose infection, which more closely resembles the natural dynamics of infection in humans[12].

Before infection at 12 weeks, all experimental groups consuming the HFD significantly increased their basal start weight from 1 or by 4 weeks as compared with the equivalent mice on the CD (Fig. 1A and 1B). Importantly, the establishment of HFD-induced obesity was maintained at comparable levels in both naïve and infected groups following both infection regimes (Fig. 1A–C). In agreement, subcutaneous adipose pads showed an increase in mass following high fat-induced obesity, unperturbed by a single low-egg dose infection of *T. muris* (Supplementary Fig. 1A). Obesity is associated with the formation of crown-like structures within adipose tissue[13], which were observed to a similar extent in mesenteric adipose from naïve and infected HFD-fed mice (Fig. 1D). These initial observations suggested that low-dose *T. muris* infection, produced by both single and trickle infection regimes, had little effect on diet-induced obesity.

Previous studies in obese mice showed that infection with *Nippostrongylus brasiliensis* or *Heligmosomoides polygyrus bakeri* resulted in reduced body weight and improved blood glucose regulation[14,15]. These models of infection develop a strong Th2 immune response and parasite expulsion; therefore, we exam-

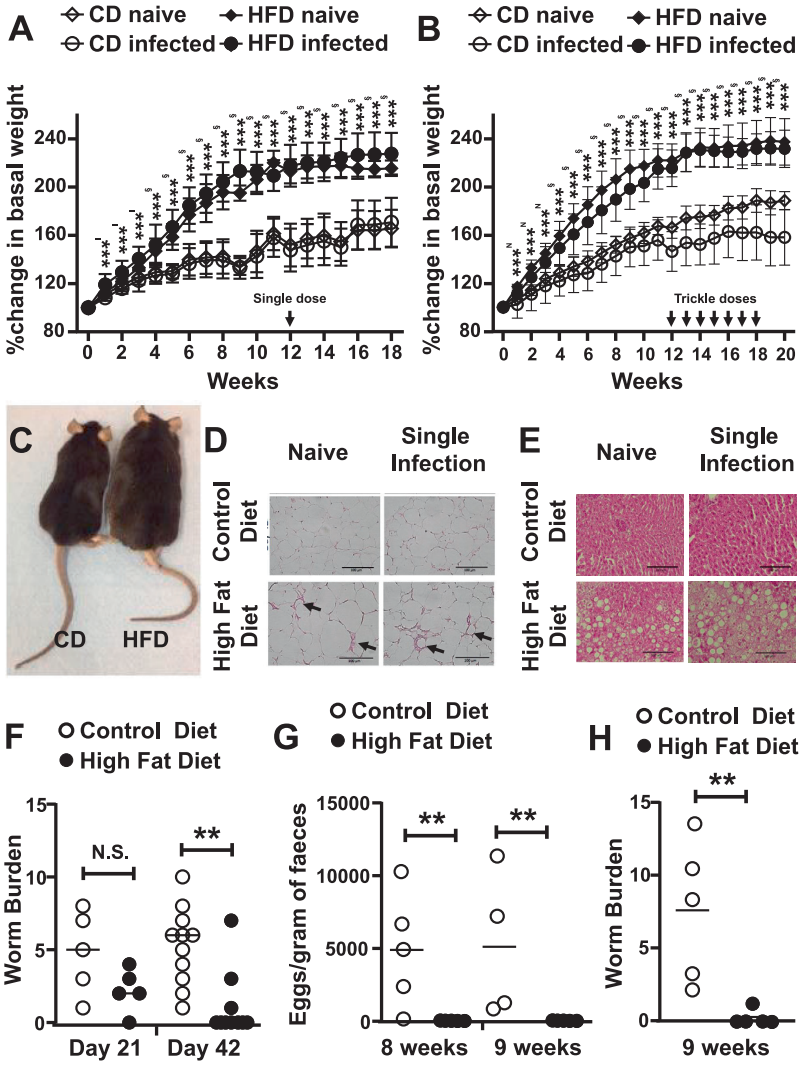

Fig. 1 HFD-induced obesity protects mice from chronic *Trichuris muris* infection. (A and B) Percentage change in basal start weight in wild-type C57BL/6 mice receiving 12 weeks of CD or HFD before a (A) single or (B) trickle low-dose *T. muris* infection (arrows indicate dose strategy). (C) Representative image of macroscopic weight gain in single dose-infected mice following 12 weeks on CD/HFD. (D and E) Representative hematoxylin/eosin-stained histology images of (D) mesenteric lymph node adipose and (E) liver in naïve and single low dose-infected mice at day 42 after infection following 12 weeks CD/HFD; arrows indicate adipose cell crowning. (F and H) Worm burdens counted from the cecum and proximal colon in mice following a CD/HFD after receiving a (F) single or (H) trickle low-dose infection. (G) Fecal egg counts at weeks 8 and 9 following a trickle *T. muris* infection in mice on CD/HFD. Data (n = 4–11 mice per group) are from two to three independent experiments performed. *, $p < 0.05$; †, $p < 0.01$; ‡, $p < 0.005$, §, ||, and ¶ indicates significant weight change between CD and HFD of infected, naïve, or both groups, respectively; N.S. via Bonferonni's multiple comparisons following (F) analysis of variance and (G) Mann-Whitney U test (A), (B), and (H) for indicated comparisons between groups. CD = control diet; HFD = high-fat diet; N.S. = not significant.

ined if a Th1 driving chronic *T. muris* infection[8,16] was able to influence obesity-driven metabolic disease. As expected, HFD-induced obesity significantly increased naïve nonfasted blood glucose as compared with naïve CD-fed mice, but both the single dose- and trickle dose-infected HFD mice presented no hyperglycemia as compared with the equivalent CD cohort (Supplementary Fig. 1B). However, although there was a trend for increased islet area of the pancreas following an HFD, indicative of early diet-induced insulin resistance[17], we saw no alterations in islet size in any of the diet or infection cohorts (Supplementary Fig. 1C). After the establishment of diet-induced obesity, the liver of the animals appeared paler (Supplementary Fig. 1D) and histology demonstrated that mice had developed fatty livers and hepatic steatosis in both infected and naïve cohorts as compared with CD equivalent cohorts (Fig. 1E), as well as elevated serum liver function markers (Supplementary Figs. 1E and 1F). Collectively, these data indicate that low-dose infection with *T. muris* alleviates the hyperglycemia that obesity induces at the time point examined but does not protect from obesity itself or metabolic disease.

We next assessed how HFD-induced obesity may influence the chronicity of infection by examining worm burdens in the cecum and proximal colon. Forty-two days after a single low-dose infection, mice on an HFD had significantly fewer worms than those on a CD, with the majority able to fully expel (Fig. 1F). Importantly, there was no significant difference at 21 days after infection, suggesting comparable establishment (Fig. 1F). Parasite egg levels were examined in the feces of trickle infected mice at 8/9 weeks, but none were detected from mice on an HFD as compared with those infected on the CD (Fig. 1G). Finally, at the termination of the trickle experiments (11 weeks), we again observed a significant reduction in worm burdens in mice on an HFD compared with those on a CD, with the majority of the mice on an HFD having fully expelled their worms (Fig. 1H). Strikingly, these data show that HFD-induced obesity results in acquired resistance to infection in marked contrast to CD-fed mice, which developed chronic infections in both single and trickle low-dose infections of *T. muris*.

HFD-induced obesity switches the immune response to *T. muris* from a susceptible Th1 to a resistant Th2 profile

We next assessed the anti-*T. muris* response, and as expected, both the single- and trickle-dose infected mice on a CD demonstrated a strong Ig (immunoglobulin) G2c and minimal IgG1 response (Fig. 2A and 2B), IgG2c production being under the tight control of the Th1-specific cytokines interferon (IFN)-gamma (IFN-γ)[18]. However, on examining the infected mice on an HFD, this was significantly different in both infection regimes, with increased IgG1, as well as IgE, and reduced IgG2c parasite-specific antibody responses as compared with CD-fed animals (Fig. 2A and 2B; Supplementary Fig. 2A).

The switch in antibody isotypes in the HFD-fed mice strongly suggests they were producing a Th2 immune response, essential for expelling *T. muris*[19]. We, therefore, examined *T. muris* antigen-specific cytokine responses in the gut-draining mesenteric lymph node. Interestingly, we saw a switch from a Th1 to a Th2 cytokine profile in the HFD-fed single low dose-infected mice, with a significant decrease in IFN-γ and significant increases in Th2 cytokines as compared with CD-fed animals (Fig. 2C–F). Furthermore, we detected significant decreases in IL-6 and tumor necrosis factor alpha (TNF-α) in the single-dose infection of HFD mice as compared with the CD-fed animals (Fig. 2G and 2H), with no significant differences in IL-17 or IL-10 (Supplementary Figs. 2B and 2C). There were no significant differences in any of the cytokines analyzed in the HFD-fed trickle dose-infected mice as compared with the CD animals (Supplementary Figs. 2D–K). This is suggestive that the local cytokine response to the helminth had already subsided following worm expulsion at least 2 weeks before examination.

We therefore focused on the single-dose experiments and sought to identify CD4+ lamina propria T-cell cytokine production, as these cells are essential for *T. muris* parasite expulsion[20]. Indeed, despite low-level IL-13 production, there were significantly decreased IFN-γ+ CD4+ T-cell numbers in infected mice on an HFD as compared with those on a CD, the latter displaying elevated levels of IFN-γ characteristic of chronic infection (Fig. 2I and 2J). Moreover, bromodeoxyuridine (BrdU) pulse-chase labeling of epithelial cells suggested an increase in epithelial cell turnover in the HFD-fed mice as compared with the CD-infected animals at day 21 after infection (Fig. 2K), a known IL-13 driven expulsion mechanism[21,22]. Collectively, these data indicate that an HFD modulates the CD4+ T-cell response, allowing a classical Th2-driven expulsion of a normally chronic-infective dose of *T. muris*.

HFD drives increased levels of adipose-derived IL-33 and CD4+ T-cell ST2 expression, but on a conventional diet, the IL-33-ST2 axis is dispensable for the expulsion of a high-dose *T. muris* infection

To further uncover the mechanism by which HFD-induced obesity promotes Th2 antigen-specific expulsion of a low-dose *T. muris* infection, we next turned our attention to alarmins, tissue-specific cytokines that have been identified as key initiators of Th2 immunity to helminth infection[9]. Thymic stromal lymphopoietin (TSLP), an epithelial alarmin important in the generation of protective Th2 immune responses against *T. muris*[23], showed no differences in the cecal transcript at day 21 after infection in HFD mice as compared with those on a CD (Supplementary Fig. 3A). We next examined tuft cell numbers, recently identified as key producers of IL-25, a key alarmin in driving goblet cell hyperplasia and helminth expulsion[24]. There was no significant tuft hyperplasia within the large intestinal epithelium in HFD-infected mice as compared with CD-fed animals (Supplementary Figs. 3B and 3C). However, there was significantly increased goblet cell hyperplasia in the HFD-infected mice as compared with their equivalent naïve HFD-fed animals, unlike in the CD-mice cohort (Supplementary Figs. 3D and 3E).

IL-33 is another key alarmin that can be produced from several tissues, including fat-associated lymphoid cluster stroma[25,26]. In mice fed an HFD, although we could not detect any *Il33* transcript from intestinal tissue, examining IL-33 cytokine production normalized to incubated tissue weight of the gonadal, mesenteric, and omental adipose depots demonstrated similar IL-33 production from all depots in HFD- and CD-fed animals at day 21 after infection (Supplementary Fig. 3F). Given the observed increased weight in fat depots in HFD-fed mice, this would result in overall increased cavity levels of IL-33. Next, we, therefore, examined the expression of the IL-33 receptor ST2 on CD4+ T cells, a well-established driver of Th2 responses[27–29], following HFD. Despite no observed increase in ST2 expression in the previously reported colonic protective ST2+ Foxp3+ population[30], we did see a small increase in ST2 expression in the non-Foxp3+ population of CD4+ T cells in

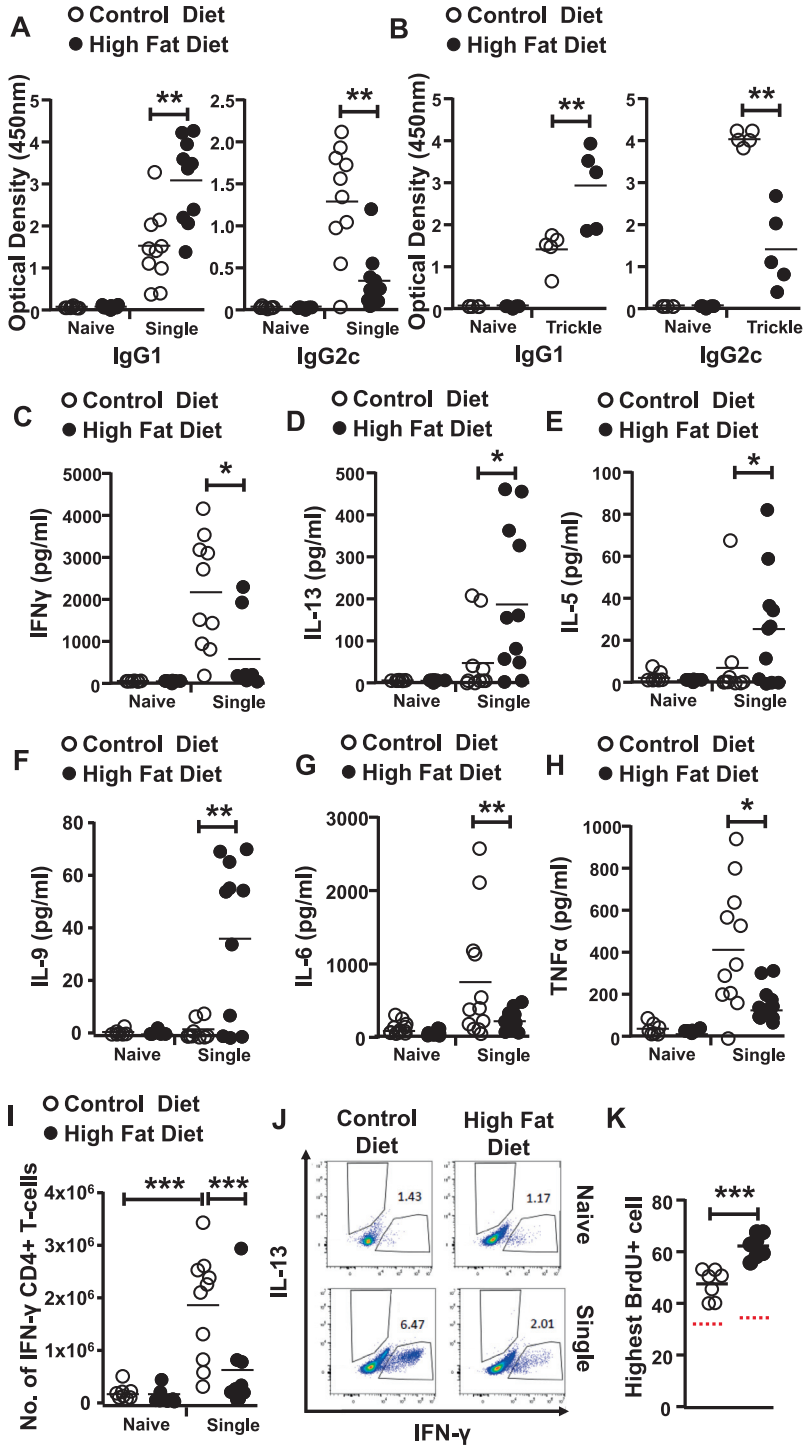

Fig. 2 HFD-induced obesity drives a Th2 immune response in mice following a low-dose *Trichuris muris* infection. (A and B) Serum parasite-specific IgG1 and IgG2c antibodies from wild-type C57BL/6 mice receiving 12 weeks of CD or HFD before a (A) single or (B) trickle low-dose *T. muris* infection. (C–H) Cytokine production from mesenteric lymph node cells of mice restimulated with parasite antigen at day 21 after infection following CD/HFD for 12 weeks, detected via cytometric bead array. (I) Total cell number and (J) representative plots of IFN-γ positive lamina propria lymphocytes CD4+ T cells via intracellular flow cytometry. (K) Percentage height of most advanced BrdU+ epithelial cell 12 hours post pulse-chase in 20 randomly selected cecal crypt units assessed via immunohistochemistry at day 21 after infection following CD/HFD for 12 weeks before a single low-dose infection, naïve mean indicated via red dashed lines. Data (n = 4–11 mice per group) are from two to three independent experiments performed. *, $p < 0.05$; †, $p < 0.01$; ‡, $p < 0.005$; N.S. via Bonferonni's multiple comparison following (A–I) analysis of variance or (K) Mann-Whitney U test for indicated comparisons between groups. BrdU = bromodeoxyuridine; CD = control diet; HFD = high-fat diet; IFN = interferon; Ig = immunoglobulin; IL = interleukin; N.S. = not significant.

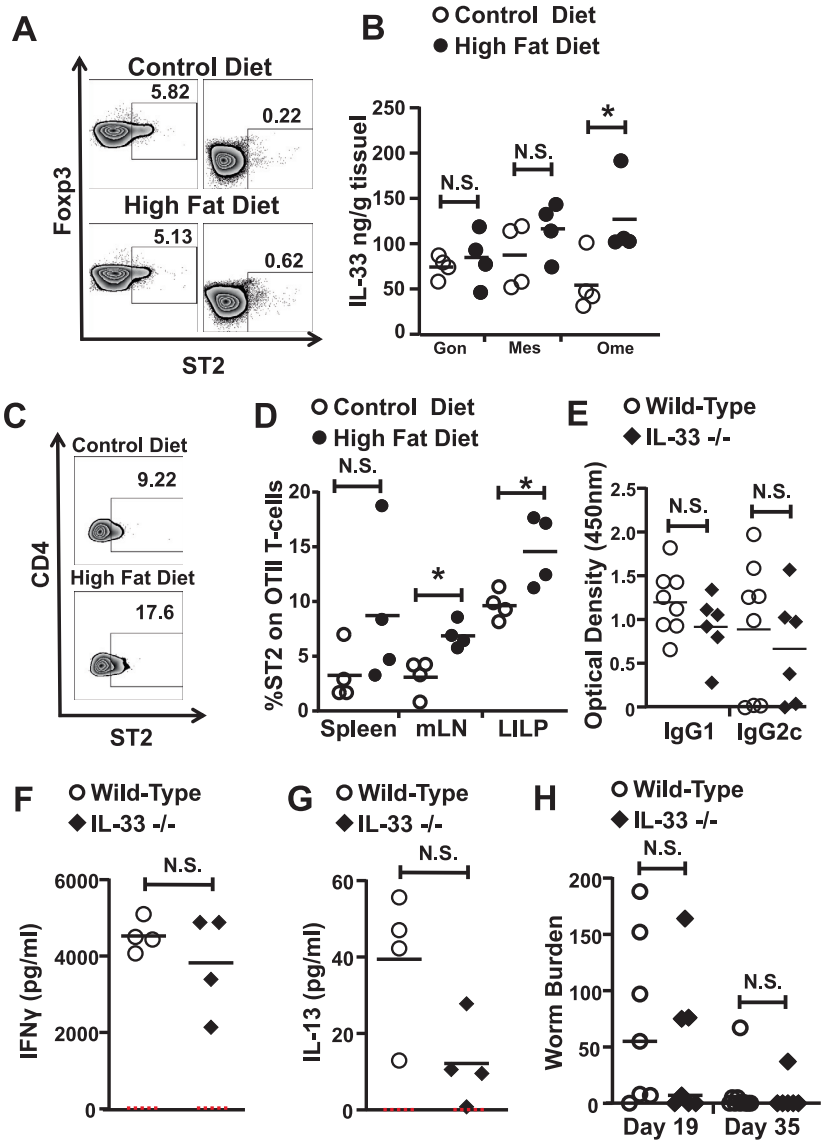

Fig. 3 HFD increases the expression of ST2 on CD4+ T cells, but the IL-33-ST2 axis is not necessary for the expulsion of a high-dose *Trichuris muris* infection in mice on a control diet. (A) Representative flow cytometry plots for ST2 expression in LILP CD3+CD4+ T cells isolated from wild-type C57BL/6 mice following 12 weeks on CD or HFD, left panels represent Foxp3+ Tregs, whereas right panels represent Foxp3- T cells. Data (n = 4 mice per group) are from two independent experiments performed. (B) IL-33 levels from normalized weight gonadal, mesentery, and omentum adipose deposits from naïve OTII/RAG−/− mice fed CD or HFD over the course of 14 weeks. Levels determined via enzyme-linked immunosorbent assay following 2-hour incubation in RPMI. CD3+CD4+ T cells examined for ST2 expression via flow cytometry, (C) representative plots, and (D) means for indicated tissues from naïve OTII/RAG−/− mice fed CD or HFD over the course of 14 weeks. Data (n = 4–8 mice per group) are from two independent experiments performed. IL-33−/− and wild-type littermate control mice received a single high-dose *T. muris* infection and were examined for (E) serum parasite-specific IgG1 and IgG2c antibodies at day 35 after infection. Cytokine production from mesenteric lymph node cells of mice restimulated with parasite antigen at day 19 after infection; (F and G) naïve mean indicated via red dashed lines, detected via cytometric bead array and (H) worm burdens in the cecum and proximal colon. Data (n = 6–8 mice per group) are from two independent experiments performed. *, $p < 0.05$; †, $p < 0.01$; ‡, $p < 0.005$; N.S. via Bonferonni's multiple comparison following (H) analysis of variance or (B and D–G) Mann-Whitney U test for indicated comparisons between groups. CD = control diet; HFD = high-fat diet; IFN = interferon; Ig = immunoglobulin; IL = interleukin; LILP = lamina propria lymphocytes; N.S. = not significant.

the HFD-obese mice as compared with the CD (Fig. 3A). In order to assess whether the increased ST2 expression occurred on naïve T cells, we placed OTII RAG−/− mice that were naïve to ovalbumin (OVA) antigen and naturally lacked Foxp3+ regulatory T cells on an HFD for 14 weeks. OTII RAG−/− mice on an HFD gained significantly more weight from week 3 as compared with CD-fed mice and continued this increase in significance (Supplementary Fig. 3G). This weight gain in HFD OTII RAG−/− mice was accompanied by increased IL-33 cytokine release from omental but not mesenteric or gonadal adipose as compared with CD-fed animals (Fig. 3B). Although baseline CD4+ T-cell ST2 expression was increased in the OTII model compared with

C57BL/6 WTs, we again saw a significant increase in CD4+ T-cell ST2 expression within the lamina propria lymphocytes (LILPs) following an HFD (Fig. 3C and 3D), whereas a significant increase was also seen in mesenteric lymph node (mLN) but not splenic CD4+ T cells (Fig. 3D).

Importantly, exogenously administered IL-33 can drive *T. muris* expulsion if given during the early stages of infection in WT mice that would normally develop a chronic *T. muris* infection[31]. Such treatment did not drive *T. muris* expulsion in severe combined immunodeficiency (SCID) mice, highlighting the importance of adaptive immunity in these IL-33-mediated effects[31]. To delineate the role of IL-33, we examined anti-*T. muris* responses in IL-33−/− mice following a Th2 inducing high-dose infection regime while under a CD. Interestingly, there were comparable parasite-specific IgG1/IgG2c antibody responses as compared with WT-littermate controls (Fig. 3E). We found no significant change in IL-13 or IFN-γ parasite antigen-specific mLN cytokine responses as compared with WT-littermate controls (Fig. 3F and 3G), indicating a parasite expelling Th2 immune response even when IL-33 is completely lacking. Indeed, IL-33−/− mice were fully able to expel a high dose of *T. muris* eggs at a comparable rate to WT-littermate controls (Fig. 3H). Collectively, these data indicate that IL-33 is not required for expulsion of *T. muris* from WT mice fed a CD, but when levels are elevated, the IL-33/ST2 axis possesses the potential to drive Th2 immune-dependent expulsion[31].

HFD as opposed to obesity drives increased CD4+ T-cell peroxisome proliferator-activated receptor-gamma (PPAR-γ) and ST2 expression as well as *T. muris* expulsion

As HFD has recently been shown to increase the expression of PPAR-γ[32], whose action is critical for CD4+ T-cell ST2 expression in response to IL-33[33], we examined PPAR-γ expression in OTII RAG−/− mice following a short-term HFD. We saw significantly increased expression of *Pprag* transcript in mLN and LILP CD4 + T cells but not splenic (Fig. 4A). To further evaluate whether an HFD itself was driving increased ST2 expression, we examined cohorts of CD-fed, aged, naturally obese C57BL/6 mice. Interestingly, we saw no significant difference in CD4+ *Pprag* transcript, ST2 expression or, following low-dose infection, differences in IgG1/2c ratio in these obese mice as compared with similarly aged mice, which remained nonobese (Fig. 4B–D). Meanwhile, mice fed a short-term HFD just before infection, which were not obese at the time of sacrifice, had increased *Pprag* transcript in CD4+ T cells, as well as ST2 expression, and had a significant difference in IgG1/2c parasite-specific antibodies (Fig. 4B–D). Moreover, comparing worm burdens in the cohorts demonstrated the obese, aged mice had no heightened ability to expel a low-dose *T. muris* infection, whereas those on the short-term HFD before infection completely cleared the parasite (Fig. 4E). We next isolated LILP CD4+ T cells from OTII RAG−/− mice and incubated them with OVA-primed dendritic cells. As previously reported *in vitro*, the addition of IL-33 was able to drive significant ST2 expression as compared with controls[29] (Fig. 4F). Moreover, treating cells with high-fat media was also able to significantly increase ST2 expression, independent of IL-33 in a process dependent on PPAR-γ (Fig. 4F). Finally, we assessed the direct importance of PPAR-γ in driving increased ST2 expression in LILP T cells *in vivo* via the use of the antagonist GW9662 during short-term HFD in OTII RAG−/− mice. We again saw a significant increase in LILP CD4+ T-cell ST2 expression as compared with CD-fed animals, but this increase was completely prevented on an HFD following PPAR-γ inhibition (Fig. 4G and 4H).

Collectively, these data indicate that an HFD rather than obesity itself is key in the phenotype of fat-induced expulsion of a low-dose *T. muris* infection, and this correlates with increased PPAR-γ and IL-33 receptor ST2 CD4+ T-cell expression.

CD4+ T cells are primed to respond to IL-33 following HFD, and diet-induced *T. muris* expulsion is absent when CD4+ T cells lack the ST2 receptor

In order to assess whether increased ST2 expression on naïve CD4+ T cells was functional, we isolated LILP CD4+ T cells from HFD- or CD-fed OTII RAG−/− mice and incubated them with OVA-primed dendritic cells. We saw minimal IL-13 production from both HFD and CD LILP CD4+ T cells incubated with vehicle alone, but both populations were equally able to produce IL-13 following IL-4 stimulation (Fig. 5A). Incubation with IL-33 produced a significant increase in IL-13 expression in CD4+ T cells from HFD-fed mice as compared with CD-fed animals (Fig. 5A and 5B). This increased IL-13 production in response to IL-33 was also seen in mLN CD4+ T cells but not observed in splenocytes (Supplementary Figs. 4A and 4B). Collectively, these data demonstrate that an HFD increases the expression of the ST2 receptor on CD4+ T cells, enabling increased IL-13 production in response to IL-33 alarmin stimulation *ex vivo*.

To test the importance of HFD-induced ST2 CD4+ T-cell expression on nematode expulsion, we utilized the small intestinal dwelling helminth *H. polygyrus bakeri*, which naturally produces several molecules that inhibit the IL-33/ST2 signaling axis during infection[34]. Before infection, mice on an HFD gained significantly more weight than those on a CD commencing from week 2 of the diet (Supplementary Fig. 4C), whereas trickle infection did not significantly affect body weight, with HFD mice maintaining a consistently significant weight (Supplementary Fig. 4C) and fat pad mass (Supplementary Fig. 4D) as compared with naïve equivalent cohorts following infection. Unlike following *T. muris* infection, there was no alteration in the key IL-13/IFN-γ parasite-specific cytokine profiles in the HFD-fed infected mice as compared with CD-fed animals (Supplementary Figs. 4E and 4F), whereas we saw minimal responses in both CD- and HFD-fed naïve animals. The nonsignificant alterations in cytokines were also mirrored in comparable levels of parasite-specific IgG1 between HFD-fed mice and equivalent CD controls (Supplementary Fig. 4G). Importantly, we saw no significant difference in the expulsion of a trickle dose of *H. polygyrus bakeri* in mice fed an HFD as compared with CD-fed animals (Supplementary Fig. 4H). Taken together, these data suggest that *H. polygyrus bakeri* modulation of the IL-33/ST2 axis may interfere with the HFD-induced regulation of the Th2 response.

In order to test the specific mechanistic importance of HFD-induced ST2 CD4+ T-cell expression in driving *T. muris* expulsion *in vivo,* we transferred WT or ST2−/− purified CD4+ T cells (Supplementary Fig. 4I) into RAG−/− mice 2 weeks before commencing a CD/HFD and then infected with a *T. muris* low dose a further 2 weeks later. Mice that had received the ST2−/− CD4+ T cells displayed weight loss at day 14 after infection, so all experimental cohorts returned to a CD for 9 days before returning to an HFD (Supplementary Fig.4J). Despite the intermittent HFD regime, transferred WT CD4+ T cells again had significantly increased expression of ST2 in the HFD cohort as compared with WT CD4+ T cells transferred into animals on a CD (Fig. 5C).

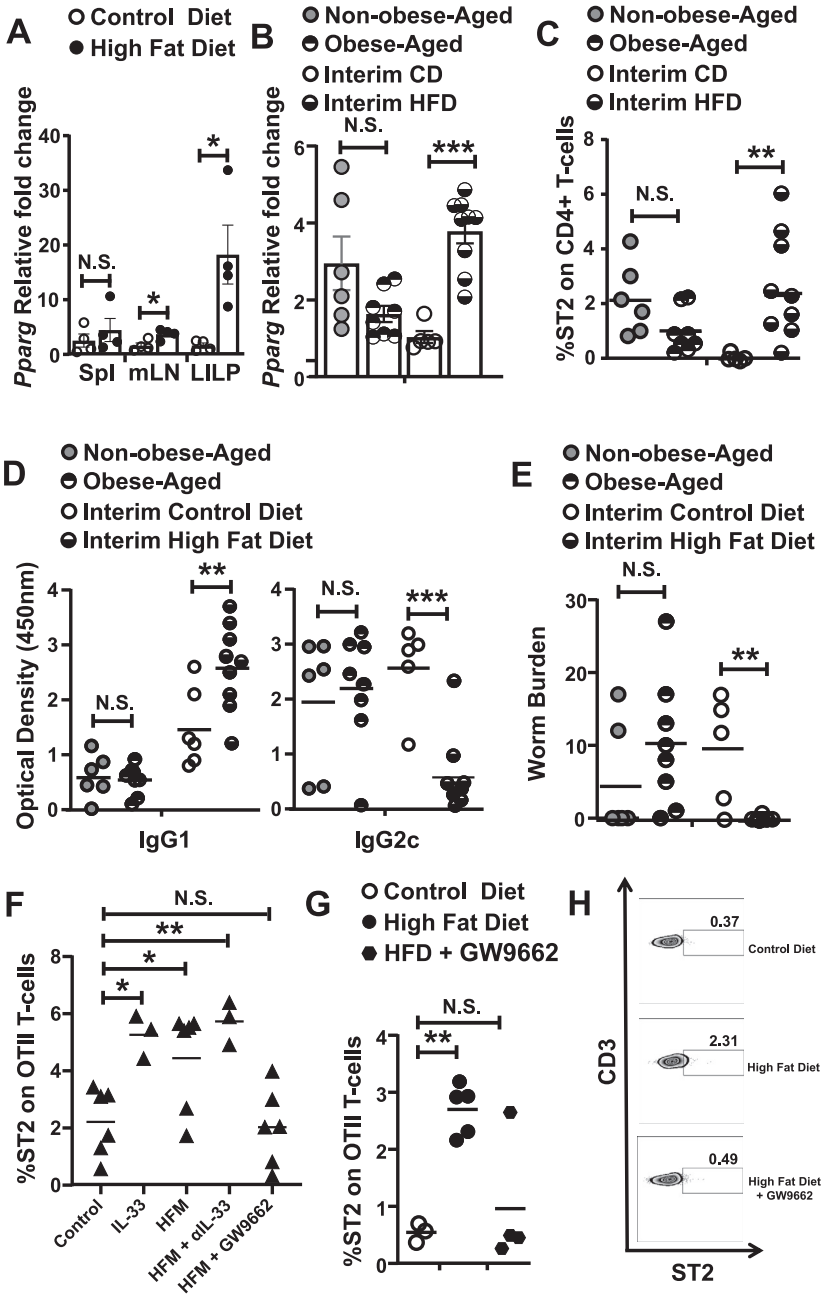

Fig. 4 HFD but not obesity increases the expression of PPAR-γ and ST2 on CD4+ T cells. OTII RAG−/− mice received an HFD for 2 weeks before sorting LILP, mLN, and splenic CD4+ T cells and isolating RNA via Qiagen RNA kit. (A) Complementary DNA was prepared from the RNA and the expression of *Pparg* relative to the control gene *HPRT* (hypoxanthine phosphoribosyltransferase) was compared using a quantitative polymerase chain reaction. Aged obese/nonobese mice received a low-dose *Trichuris muris* infection and were compared with mice receiving HFD/CD 2 weeks before a low-dose *T. muris* infection and examined at day 42 after infection. (B) Expression of *Pparg* relative to the control gene *HPRT* was compared using a quantitative polymerase chain reaction from sorted mLN CD4+ T cells following RNA isolation via Qiagen RNA kit and complementary DNA preparation. (C) ST2 expression on CD3+CD4+ T cells from mLN via flow cytometry, (D) serum parasite-specific IgG1 and IgG2c antibodies via enzyme-linked immunosorbent assay, and (E) worm burdens in the cecum and proximal colon. Data (n = 4–8 mice per group) are from two independent experiments performed. A total of 2×10^5 CD11c+ dendritic cells were incubated with 0.8 μg/ml 323-339 ovalbumin and incubated with LILP cells from OTII/RAG mice. (F) ST2 expression on CD3+CD4+ T cells was assessed after 5 days in the presence of 20 ng/ml IL-33, 2% high-fat lipid media, 1 μg/ml anti-IL-33, or 10 μm PPARg inhibitor GW9662 as indicated. Data (n = 3–6 mice per group) are from three independent experiments performed. OTII RAG−/− mice received an HFD for 3 weeks and received either vehicle or 1 mg/kg of GW9662 every 3 days via intraperitoneal injection. (G) ST2 expression on CD3+CD4+ T cells from LILP and (H) representative plots via flow cytometry. Data (n = 3–5 mice per group) are from two independent experiments performed. *, $p < 0.05$; †, $p < 0.01$; ‡, $p < 0.005$; N.S., not significant via Bonferonni's multiple comparison following (B–G) analysis of variance or (A) Mann-Whitney U test for indicated comparisons between groups. CD = control diet; HFD = high-fat diet; IFN = interferon; Ig = immunoglobulin; IL = interleukin; LILP = lamina propria lymphocytes; mLN = mesenteric lymph node; N.S. = not significant; *Pparg* = Peroxisome proliferator-activated receptor-gamma.

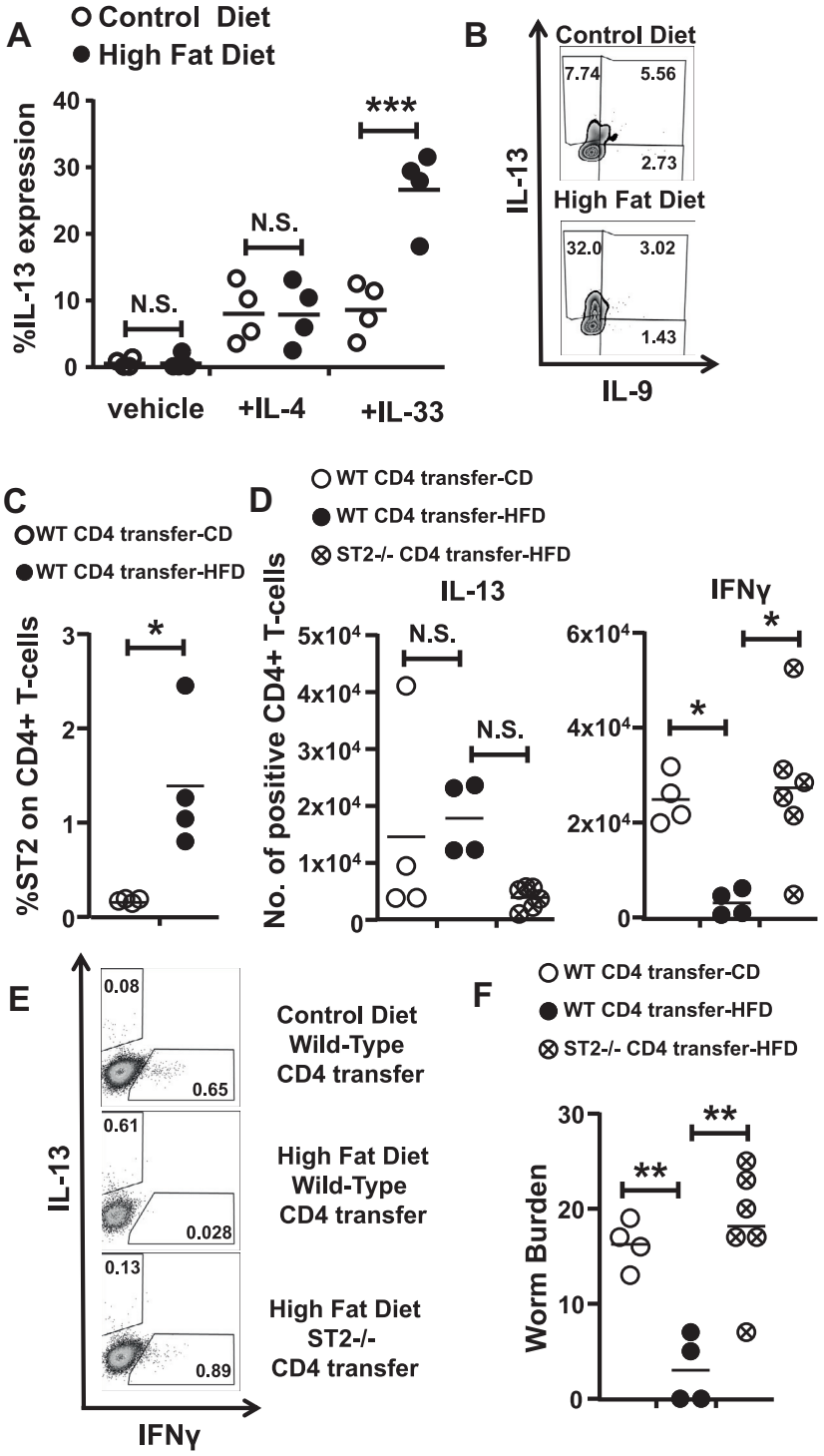

Fig. 5 HFD increases functional ST2 expression priming CD4+ T cells for the expulsion of a low-dose *Trichuris muris* infection. A total of 2×10^5 CD11c+ dendritic cells were incubated with 0.8 μg/ml 323-339 ovalbumin and incubated with lamina propria lymphocytes cells from OTII/RAG mice fed with CD or HFD for 14 weeks. Cytokine expression was assessed by flow cytometry after 4 days in the presence of vehicle, 5 ng/ml IL-4, or 20 ng/ml IL-33. (A) Percentage IL-13 expression in OTII T cells and (B) representative flow cytometry plots. Data ($n = 4$ mice per group) are from two independent experiments performed. RAG−/− mice received 5×10^6 CD4+ T cells from WT or ST2−/− mice via intraperitoneal injection 2 weeks before receiving an intermittent CD/HFD and single low-dose *T. muris* infection. (C) Percentage ST2 expression on transferred WT CD3+CD4+ T cells from mLN at day 32 after infection following CD/HFD via flow cytometry. (D) Total cell number and (E) representative plots of IFN-γ- and IL-13-positive mLN CD4+ T cells via intracellular flow cytometry. (F) Worm burdens in the cecum and proximal colon. Data ($n = 4$–6 mice per group) are from two independent experiments performed. *, $p < 0.05$; †, $p < 0.01$; ‡, $p < 0.005$; N.S. via Bonferonni's multiple comparison following (A, D, and F) analysis of variance and (C) Mann-Whitney U test for indicated comparisons between groups. CD = control diet; HFD = high-fat diet; IFN = interferon; Ig = immunoglobulin; IL = interleukin; mLN = mesenteric lymph node; N.S. = not significant; WT = wild-type.

Although not significant, intracellular IL-13-producing CD4+ T cells numbers were the highest in the mLN of mice receiving WT cells and an HFD, as opposed to those on a CD or an HFD with ST2−/− cells (Fig. 5D). Moreover, mice on a CD receiving WT CD4+ T cells and mice on an HFD receiving ST2−/− CD4+ T cells had significantly higher numbers of IFN-γ producing cells in the mLN as compared with those animals receiving WT cells on an HFD (Fig. 5D and 5E). This culminated in a significantly reduced or absent worm burdens in mice receiving WT CD4+ T cells placed on an HFD as compared with those on a CD (Fig. 5F), whereas mice on an HFD receiving ST2−/− CD4+ T cells failed to expel a low-dose *T. muris* infection (Fig. 5F). Collectively, these data suggest an HFD drives an increase in expression of the IL-33 receptor ST2 on CD4+ T cells, which primes gut-associated T cells for Th2 polarization and helminth expulsion.

DISCUSSION

In this study, C57BL/6 mice were fed an HFD for 12 weeks to induce obesity, and consistent with published literature, mice became overweight and showed signs of obesity such as hyperglycemia and fatty liver[17]. Using single low-dose or trickle infection of *T. muris* eggs, we observed that both infection regimes prevented HFD-induced increases in nonfasting glucose levels. However, there was no significant reduction in body weight nor signs of metabolic disease in contrast to previous reports of mice infected with *H. polygyrus bakeri*[14] or *N. brasiliensis*[15], where strong Th2 responses increase adipose eosinophilia modulating metabolic homeostasis[14]. Our results suggest that low-dose *T. muris* infection does not exert such a strong influence on the pathology of HFD-induced obesity, suggesting that the transient Th2 response seen here during HFD *T. muris* infection may not be sufficient to cause these major metabolic alterations as compared with stronger type 2-inducing helminth infections. Remarkably, however, the Th2 response induced in HFD mice was associated with loss of worms from the intestine—a single low-dose infection mostly fully expelled by day 42 and trickle infections cleared by week 9, time points usually associated with heavy worm burdens[8,12].

The immune-mediated expulsion of *T. muris* is classically dependent on CD4+ T cells[9], and there is little change in ILC2 numbers following *T. muris* infection, their depletion having no impact on resistance[12]. It is well established that resistance to *T. muris* is associated with the increased production of CD4+ T-cell-derived Th2 cytokines with IL-13 being essential[35]. IFN-γ is associated with susceptibility[8], whereas IL-13-dependent epithelial turnover acts as an effector mechanism of expulsion[21,22]. This work demonstrated a significant increase in the CD4+ Th2 response (and a decrease in the CD4+Th1 response) in infected mice on an HFD as compared with mice fed with a CD. The observation that HFD-induced worm expulsion was correlated with a classical Th2 response and increased epithelial turnover was quite surprising given the general consensus and reporting that obesity is a pro-inflammatory-like state. Indeed, several reports identify IFN-γ as a cytokine involved in the pathogenesis of obesity with IFN-γ producing adipose T cells associated with metabolic disease[36,37]. However, we observed significant increases in parasite-specific IL-13, IL-5, and IL-9, as well as significantly reduced IFN-γ + CD4 T cells in the lamina propria and increased epithelial turnover in HFD-infected animals. Interestingly, during Leishmaniasis, an HFD causes reduced protective Th1-driven responses, leading to enhanced Th2-driven pathology[38], demonstrating HFD-driven alteration of the expected Th1 response in another infection model.

We next examined early alterations in alarmins, tissue-specific cytokines that have been identified as key initiators of Th2 immunity to helminth infection[9]. TSLP promotes the Th2 immune response and is elevated in the early stages of *T. muris* infection in resistant mice[31], and although an HFD has also been associated with elevated levels of TSLP in the serum[39], we observed no difference in *Tslp* transcript in the cecum between HFD- and CD-infected mice. Tuft cells, potent sources of IL-25 and thus key drivers of ILC2 expansion, exist at very low frequency in the cecum, and in parallel to other studies[12], we did not see any alteration in their numbers following HFD alone or in combination with *T. muris* infection.

We turned our attention to IL-33, as a potent driver of IL-13 production and type 2 immune responses. Interestingly, IL-33 signaling is not required for resistance to *T. muris* during a CD as demonstrated by our data showing IL-33−/− mice are fully able to expel a high dose of *T. muris*. This corroborates previous studies where IL-1AcP−/−[40] and MyD88−/− mice[41] all expel *T. muris* efficiently. However, the administration of exogenous IL-33 to mice that would normally progress to chronic infection induces robust worm expulsion[31], suggesting that the IL-33/ST2 receptor axis can be activated and lead to *T. muris* clearance. We observed a significant increase in IL-33 secretion within omental adipose depots following an HFD in naïve RAG OTII mice. The source of IL-33 in mesenteric and omental adipose is potentially the fat-associated lymphoid cluster stroma, as this has previously been reported to be a key source of IL-33 during parasite infection[25,26,42]. Moreover, IL-33 has been shown to increase IL-13 production from adipose, which is also reflected in raised serum levels *in vivo*[43], whereas obesity itself is associated with increased IL-33 production[44]. On examining levels of the IL-33 receptor ST2, we observed a significant increase in ST2 expression on CD4+ T cells in mice following an HFD, and importantly, this occurred before infection. IL-33 itself can drive increased ST2 expression[29], but we demonstrate *in vitro* that high-fat lipid media could also induce ST2 in an IL-33-independent fashion. This potentially suggests that the signal *in vivo* driving increased ST2 expression on CD4+ T cells could be both directly diet driven and via the resulting increase in IL-33. Significantly, we show that these ST2 high-expressing T cells were primed to respond to IL-33 *ex vivo* and produced heightened levels of IL-13 *in vivo*. Interestingly, the increase in ST2 expression correlated with worm expulsion, as it was absent in aged, obese mice, which demonstrated no ability to expel a chronic infection but was present in short-term HFD-fed mice that were not obese but again able to expel a chronic infection. This phenotype, therefore, appears to be due to diet rather than obesity itself, suggesting that the high lipid content rather than increased IL-33 could be the key factor driving increased ST2 or conversely that rapid adipose expansion in HFD-fed mice, absent in gradual weight gain in old obese mice, increases cellular stress and alarmin release.

HFD has recently been shown to increase the expression of the lipid-activated transcription factor PPAR-γ[32]. Moreover, *pparg* is selectively upregulated in CD4+ T cells that produce IL-13[45], and its action is critical for their expression of ST2 and response to IL-33[33]. Indeed, the increased ST2 expression seen on CD4+ T cells following *T. muris* infection on an HFD, and baseline levels in aged, obese mice, correlated with increased and stable PPAR-γ activity in HFD-fed and aged, obese mice, respec-

tively. Furthermore, the antagonism of PPAR-γ prevented both lipid media and HFD-induced increases of ST2 *in vitro* and *in vivo*, respectively. This would therefore enhance responses to the elevated IL-33 produced following an HFD, and mediate worm expulsion similar to that seen following exogenous administration of IL-33[31]. A recent study examining CD4+ T-cell responses in atopic dermatitis also demonstrated HFD-driven changes in PPAR-γ expression[46], which together with our observed increased levels of specific IgE in HFD-infected mice raises a key potential impact of obesity on atopy. However, in the usually Th2-driven system of atopic dermatitis, PPAR-γ activity was seen to be reduced resulting in a more Th17 severe disease, although full ablation of PPAR-γ demonstrated expression was required to focus CD4+ T-cell polarization toward the Th2 state[46]. We did not see any differences in parasite-specific mLN IL-17 production in HFD-fed *T. muris*-infected animals, nor any changes in IL-13 levels in the Th2-driven *H. polygyrus* infection. Collectively, this indicates that HFD may influence CD4+ T-cell responses in a tissue, and initial Th polarization-driven specific context.

To examine the mechanistic importance of IL-33 in our observed phenotype of increased helminth expulsion following an HFD, we examined how an HFD would influence the expulsion of *H. polygyrus*, which naturally inhibits the IL-33/ST2 signaling axis *in vivo*[34]. Despite the HFD, we saw no alterations in antigen-specific IL-13, IFN-γ, or IgG1 levels in marked contrast to the effects seen during *T. muris* infection following an HFD. Finally, via adoptive transfer of WT and ST2−/− CD4+ T cells, we were able to demonstrate that without the presence of ST2, CD4+ T cells are unable to induce a Th2 response and expel *T. muris* following an HFD. Importantly, as previously mentioned, mice lacking IL-33 on a CD were fully able to expel a high-dose *T. muris* infection, indicating that IL-33 is not usually utilized in resistance to *T. muris*.

In conclusion, we have demonstrated that an HFD results in increased expression of the ST2 receptor on CD4+ T cells, priming for Th2 polarization and utilizing an alternative expulsion mechanism to clear a low-dose *T. muris* infection. This study emphasizes that in an infection system where CD4+ Th2 cells are key to immunity, diet can profoundly alter the capacity to generate protective immunity. The increasing prevalence of HFDs and obesity calls for more work to be done to investigate the implications of the dual burden of obesity and helminth infection and raises important questions on how obesity may influence the dynamics of multiple host infections in endemic areas.

MATERIALS AND METHODS

Animals

C57BL/6 mice (ENVIGO, Leicestershire, UK), C57BL/6 OTII RAG−/− mice, and IL-33 genetrap mice[47] (a kind gift of Dr Jean-Phillipe Girard; University of Toulouse) were maintained in individually ventilated cages on a 12-hour light or dark cycle at 22°C ± 1°C and 65% humidity. All procedures were carried out in accordance with the Home Office Animal Scientific Procedures Act (1986). Experiments were carried out at the University of Manchester/Lancaster under the project licenses 70/8127/P043A3082 or 70/8521/PP4157153, respectively. All experiments conformed to the relevant Animal Welfare and Ethical Review Body (AWERB) and Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines. All animals were humanely killed by a schedule 1 method followed by terminal exsanguination.

Diet modifications

Mice were given free access to CD (12% Kcal from fat, Research Diets Inc., NJ, USA, D16011009) or HFD (60% Kcal from fat, Research Diets Inc., NJ USA, D12492) for indicated periods of time before infection. Mice received either vehicle or 1 mg/kg of GW9662 (Tocris, Bristol, UK, 1508) every 3 days via intraperitoneal injection as indicated.

Parasites

The techniques used for maintenance and infection of *T. muris* and of *H. polygyrus* were as previously described[16,48].

Blood glucose/liver readouts

Glucose was measured from freshly drawn tail blood using an Accu-Chek Aviva blood glucose meter (Roche, Basel, Switzerland, 06988563016) from nonfasted mice. Alanine concentration was determined by a coupled enzyme assay (Sigma-Aldrich, MO, USA, MAK001).

Histology

Tissue sections were stained in hematoxylin/eosin or Schiff's reagent (Sigma-Aldrich, MO, USA; for visualizing goblet cells). Tuft cell staining was via primary antibody anti-DCAMKL1 (1:500, Abcam, Cambridgeshire, UK, Ab31704) as previously described[12]. After mounting, positive cells were enumerated in 20 randomly selected cecal crypt units (CCU) and the results were presented as the mean number of positive cells/20 CCU (±S.E.). BrdU incorporation was assessed via immunohistochemistry, using a monoclonal anti-BrdU antibody following a 12-hour pulse-chase as previously described[21].

Isolation and restimulation of immune cells

mLNs were removed from mice and disaggregated through a 100-μm sieve. Colon/cecums were excised, and LILPs were prepared as described[49]. Cells at 5×10^6 cells/ml in complete media received 50 μg/ml of parasite excretory/secretory antigen. Supernatants were collected after 24 hours, and cytokines were measured using a cytometric bead array kit (BD, NJ, USA) according to the manufacturer's instructions. For IL-33 analysis, equivalent weights of adipose samples were incubated in Roswell Park Memorial Institute Medium (RPMI; Sigma-Aldrich, MO, USA, R8758) for 2 hours and supernatant cytokine levels were detected on a mouse DuoSet enzyme-linked immunosorbent assay (R and D Systems, MN, USA, DY3626) normalized to tissue weight. A total of 2×10^5 CD11c+ Miltenyi LS column (Miltenyi, NRW, Germany) purified dendritic cells were incubated with 0.8 μg/ml 323-339 OVA (Universal Biologicals, Cambridge, UK, CS-7035) and incubated with LILP cells from OTII/RAG mice for 4–5 days in the presence of 5 ng/ml IL-4 (Peprotech, London, UK, 214-14), 20 ng/ml IL-33 (R and D, MN, USA, 3626-ML), 2% high-fat media (Lipid mixture 1; Sigma-Aldrich, Mo, USA, L0288), 1 μg/ml anti-IL-33 (R and D, MN, USA, AF3626), or 10 μm GW9662 (Tocris, Bristol, UK, 1508). For intracellular cytokine analysis, cells were incubated for 12 hours with ×1 cell-stimulation cocktail (plus protein inhibitors) (eBioscience, MA, USA, 00-4975-93). Cells were then stained with antibodies using the eBioscience Foxp3 permeabilization kit (eBioscience, MA, USA, 00-5523-00) according to the manufacturer's instructions. Cell suspensions were blocked with anti-FcγR antibodies and then labeled (see Table 1). Samples were analyzed on a Cytoflex (Beckman, CA, USA) or FACS LSRII (BD, NJ, USA).

Table 1. List of flow cytometry antibodies.

Fcγ receptor	clone 24G2; eBioscience
CD3	eBio500A2; eBioscience
CD4	clone GK1.5; eBioscience
Foxp3	clone FJK-16s; eBioscience
Interleukin-13	clone eBiol13A; eBioscience
Interferon-γ	clone XMG1.2; eBioscience
Interleukin-9	RM9A4e; Biolegend
ST2	RMST2-2; eBioscience

Cell purification and adoptive transfer

Lymph nodes and spleens were excised from WT, OTII RAG−/−, or ST2−/− mice (a kind gift from Professor Andrew McKenzie; University of Cambridge) and incubated with shaking for 20 mins at 37°C in RPMI 1640 with 0.08 U/ml liberase blendzyme 3 (Roche, Basel, Switzerland, 5401020001) or 1 mg/ml collagenase VIII and 50 U/ml DNAsel, respectively (Roche, Basel, Switzerland, 04536282001. Cells were antibody labeled before enrichment on an MA-900 multi-application cell sorter (SONY Europe, Surrey, UK). In all experiments, subset purity was >95%. RAG−/− mice received 5×10^6 CD4+ T cells via intraperitoneal injection 2 weeks before receiving an intermittent CD/HFD and single low-dose *T. muris* infection.

Serum antibody

Parasite-specific IgG1 and IgG2c were assessed via coating enzyme-linked immunosorbent assay plates with 5 μg/ml parasite excretory/secretory antigen in 0.05 M carbonate/bicarbonate buffer, pH 9.6. IgG1 and IgG2c were then detected using biotinylated rat-anti-mouse antibodies (Pharmingen, NJ, USA, clone. A85-1 and Serotec, CA, USA, 553388 respectively) diluted in PBS-Tween. For IgE, serum was stripped of IgG via sequential incubating with plate-bound rabbit-anti-mouse IgG (Biorad, CA, USA, STAR26B) and detected using biotinylated goat-anti-mouse IgE (Biorad, CA, USA, STAR110P) Titres were visualized using streptavidin peroxidase and 2,2′-Azinobis-(3-Ethylbenzo thiazoline-6-Sulfonic) (ThermoFisher, MA, USA, 37615) substrate before being read 405 nm on a VersaMax microplate reader (Molecular Devices, Berkshire, UK).

Quantitative polymerase chain reaction

Total RNA was purified from the cecal epithelial tissue using Trizol reagent according to the manufacturer's instructions (ThermoFisher, MA, USA,15596026). RNA was reverse transcribed using oligo(dT) primers (Promega, WI, USA, C1101) and complementary DNA for specific genes (see Table 2) detected using an SYBR Green qPCR Kit (Roche, Basel, Switzerland, FSUSGMMRO). Gene expression normalized to a specified housekeeping gene.

Statistics

Results are expressed as mean error bars representing ±S.E. Where statistics are quoted, two experimental groups were compared via the Mann-Whitney U test. Three or more groups were compared with the analysis of variance, with Dunnett's or Bonferroni's after test as indicated. *, $p < 0.05$; †, $p < 0.01$; or ‡, $p < 0.005$ for indicated comparisons.

AUTHOR CONTRIBUTIONS

J. J. W., R. K. G., and D. J. T. conceived the study. E. F., S. A. P. C., K. S. H., M. J. T., K. A. T., S. C., A. A. F., R. K. S., M. D., L. H. J. J., and J. J. W. initiated the study design and curated data. E. F., W. A., M. A., A. A., Y. A., L. H. J. J., D. J. T., J. J. W., and R. K. G. were involved in funding acquisition. E. F. and J. J. W. wrote the original draft. S. A. P. C., W. A., M. A., A. A., L. H. J. J., D. J. T., and R. K. G. reviewed and edited the manuscript. All authors contributed to the refinement of the study protocol and approved the final manuscript.

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DECLARATION OF COMPETING INTEREST

The authors have no competing interests to declare.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mucimm.2023.01.004.

Table 2. List of primers.

Transcript	Forward	Reverse
B-actin	5′TCTTGGGTATGGAATGTGGCA3′	5′ACAGCACTGTGTTGGCATAGAGGT3′
Hprt	GCGTCGTGATTAGCGATGATGAAC	GAGCAAGTCTTTCAGTCCTGTCCA
Tslp	5′AGCAAGCCAGTCTGTC TCGTGAAA3′	5′TGTGCCAATTCCTGAGTA CCGTCA3′
Pparg	5′CCAGCATTTCTGCTCCACAC3′	5′GATGCCTTCTTGTCAGAGTGC3′

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