Obesity aggravates contact hypersensitivity reaction in mice

Short title: Effect of obesity on CHS

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AUTHOR CONTRIBUTIONS

M.M.-S., M.S., P.K., A.S. and L.W. designed experiments, interpreted the data and wrote the manuscript. M.M.-S., P.K., K.M., A.S. and M.S., performed the research and analysed the data. G.L., histopathological examination of tissue samples. F.S.W reviewed the data and edited the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ABSTRACT

BACKGROUND: Obesity is associated with chronic, low-grade inflammation in tissues and predisposes to various complications, including inflammatory skin diseases. However, the link between obesity and contact hypersensitivity (CHS) is not fully understood.

OBJECTIVES: We sought to determine the influence of obesity on Th1-mediated CHS.

METHODS: The activity/phenotype/cytokine profile of the immune-cells was tested in vivo and in vitro. Using qPCR and fecal microbiota transplantation, we tested the role of high fat diet (HFD)-induced gut microbiotta (GM) dysbiosis in increasing the effects of CHS.

RESULTS: Exacerbated CHS correlates with an increased inflammation-inducing GM in obese mice. We showed a proinflammatory milieu in the subcutaneous adipose tissue of obese mice, accompanied by proinflammatory CD4+ T cells and dendritic cells in skin draining lymph nodes and spleen. Obese IL-17A-/-B6 mice are protected from CHS aggravation, suggesting the importance of IL-17A in CHS aggravation in obesity.

CONCLUSIONS: Obesity creates a milieu that induces more potent CHS-effector cells but does not have effects on already activated CHS-effector cells. IL-17A is essential for the pathogenesis of enhanced CHS during obesity. Our study provides novel knowledge about antigen-specific responses in obesity, which may help with improvement of existing treatment and/or in designing novel treatment for obesity-associated skin disorders.

Keywords: contact hypersensitivity, dendritic cells, high-fat-diet-induced-obesity, skin inflammation

Abbreviations:

ALN: axillary and inguinal lymph nodes, ALNC: axillary and inguinal lymph node cells, AT: adipose tissue, ATDCs: adipose tissue dendritic cells, CHS: contact hypersensitivity reaction,

DCs: dendritic cells, dDCs: dermal dendritic cells, ELNC: auricular lymph node cells, FMT: fecal microbiota transplantation, FMT HFD: fecal microbiota transplantation from HFD-fed donors, FMT ND: fecal microbiota transplantation from ND-fed donors, GM: gut microbiota, HFD: high-fat diet, HFDIO: HFD-induced obesity/ HFD-induced obese, LCs: Langerhans cells, LNC: lymph node cells, MPO: myeloperoxidase, ND: normal diet, ~~PCl: pieryl chloride,~~ scAT: subcutaneous adipose tissue, sDCs: skin dendritic cells, sLNs: skin draining lymph nodes, SPL: spleen, SPLC: splenocytes, TNCB: 2,4,6-trinitrochlorobenzene.

1. INTRODUCTION

Contact hypersensitivity reaction (CHS) is a delayed hypersensitivity to haptens. During the sensitization phase in animal models, activated keratinocytes produce proinflammatory cytokines, IL-1β and TNF-α, promoting migration and maturation of skin dendritic cells (sDCs) [1]. sDCs like Langerhans cells (LCs) and dermal dendritic cells (dDCs) present antigen during both sensitization (induction) and elicitation (effector) phases of CHS, to shape the adaptive immune response. LCs have regulatory and stimulatory functions during sensitization in CHS [2], whereas Langerin (CD207)-expressing dDCs exert only stimulatory effects [3, 4]. Increasing evidence from a number of studies suggests that effector cells that mediate CHS are IFN-γ-producing $CD4^+$T helper 1 (Th1) and $CD8^+$T cytotoxic 1 (Tc1) lymphocytes [5, 6]. Moreover, IL-17 is also required for hapten-specific T cell sensitization, especially for $CD4^+$T cells [7], whereas IL-17-producing $CD8^+$ T cell effectors provoke CHS [8].

Inflammation plays an important role in obesity, with immune cell infiltration in the adipose tissue (AT) of obese humans and mice induced by high-fat diet (HFD) [9]. $CD4^+$Th1 cells, $CD8^+$ Tc1 effector cells and macrophages were found in the infiltrates of HFD-induced obese (HFDIO) mice, whereas the number of regulatory T cells was decreased [10]. DCs in AT were found to enhance inflammation by inducing Th17 cell development [11]. HFD also causes significant changes in gut microbiota (GM), characterized by a decreased abundance of *Bacteroidetes* and a correspondingly increased abundance of *Firmicutes* [12]. Some gut bacteria promote anti-inflammatory responses, whereas others induce inflammatory reactions [13, 14]. GM dysbiosis and proinflammatory conditions in obese individuals predispose to various complications, including type 2 diabetes [12], liver diseases [15], cardiovascular diseases [16], and autoimmune disorders [17]. Recent studies have shown that obesity is a major risk factor for the development of inflammatory skin diseases [18]. Katagiri et al. demonstrated that HFDIO partially impairs skin immunity in some mouse strains [19], and Watanabe et al. reported that

nickel allergy is more common in obese patients [20]. However, there is still a significant knowledge gap in understanding the mechanism by which obesity affects skin diseases. To fill this knowledge gap, we investigated HFDIO and CHS to 2,4,6-trinitrochlorobenzene (TNCB) ~~picryl chloride (PCl)~~ in mouse models. We demonstrated that HFDIO aggravates CHS in C57BL/6 mice by altering GM composition and promoting a proinflammatory response *in vivo*. Our *in vitro* studies revealed that the proinflammatory response occurs in the skin draining lymph nodes (sLNs), in spleen (SPL), and subcutaneous adipose tissue (scAT). Moreover, we found that IL-17A plays an important role in the CHS aggravation.

2. METHODS

2.1 Mice

Wild type male C57BL/6 (H2b) mice were purchased from the Jackson Laboratory Bar Harbor, ME and maintained at Yale University School of Medicine and in the Department of Medical Biology Jagiellonian University Medical College. IL-17A-/- C57BL/6 (H2b) breeders were kindly provided by Dr. R. A. Flavell (Yale University) and the colony was expanded at Yale University School of Medicine. The procedures used in this study were approved by IACUC of Yale University and 1st Local Ethical Committee on Animal Testing in Krakow.

2.2 Sensitization and elicitation of CHS *in vivo*

Mice were sensitized by application of 150 µl of 5% TNCB ~~PCl~~ in acetone-ethanol mixture (1:3 ratio) to the shaved abdomen (positive groups). Unsensitized mice fed with HFD or ND for 8 weeks were used as negative controls. Four days later, all the mice were challenged on both ears with 10 µl of 0.4% TNCB ~~PCl~~ in olive oil-acetone mixture (1:1 ratio). The ear thickness was measured prior to testing with a micrometer (Mitutoyo, Tokyo, Japan), by an observer unaware of the experimental groups and then again at 24h after challenge. Ear

thickness was calculated as (Ear thickness [μm] 24h after challenge) - (Ear thickness [μm] before challenge). The ear swelling was expressed in μm ± SEM. Sections were examined under Olympus BX50 microscope (Olympus, Japan). Images were recorded using DP-71 digital CCD camera (Olympus,Japan) coupled to IBM PC-class computer equipped with AnalySIS-FIVE (Soft Imaging System GmbH, Münster, Germany) image analysis system.

2.3 Adoptive transfer of CHS

CHS-immune effector cells were from the donor mice, fed with HFD or ND for 8 weeks prior to sensitization with 5% TNCB ~~PCl~~. Immune cells from axillary and inguinal lymph nodes (ALN), as well as SPL were isolated 4 days after TNCB ~~PCl~~ sensitization. In brief, the minced ALN and SPL tissue were filtered through a 70 μm nylon filter, the cells were washed with PBS supplemented with 1% FBS, and centrifuged at 4°C at 300 g for 10 min. The supernatant was decanted, and the remaining cell pellets were resuspended with PBS and counted, based on trypan blue exclusion. $8x10^6$ ALNC/recipient and $16x10^6$ SPLC/recipient or $2x10^7$ ALNC+SPLC/recipient were injected *i.v.* into naive syngeneic recipients fed with ND or HFD. All the animals were challenged with 10 μl of 0.4% TNCB ~~PCl~~ in olive oil-acetone mixture (1:1 ratio) next day after cell transfer and tested for CHS 24 h later. The baseline of ear thickness was measured before cell transfer. Mice in the negative control group did not receive any cells prior to challenge and CHS test.

More information about materials and methods used in this study can be found online in the supporting information tab for this article.

3. RESULTS

3.1 Obesity exacerbates CHS in mice

To determine the effect of obesity on CHS, we fed C57BL/6 mice with HFD or normal diet (ND) for 8 weeks prior to sensitization and challenge with TNCB ~~PCl~~. As expected, HFD-fed mice gained significantly more weight (Figure 1A) and interestingly, developed enhanced CHS, indicated by ear swelling (Figure 1B, left panel, Group D vs. B) and ear weight (Figure 1B right panel, Group D vs. B), when compared with control ND-fed mice. Histological examination in HFD sensitized mice (Group D) revealed increased thickening of the edematous dermis, thickened, hyperplastic epidermis as well as significant accumulation of inflammatory cells (polymorphonuclear and mononuclear) mainly in dermis with formation of microabscesses in epidermis (Figure 1C, Group D vs. B). Moreover, we found a strikingly elevated production of IL-17A, without obvious changes in IFN-γ and TNF-α secretion, by auricular lymph node cells (ELNC) in HFD-fed mice, when compared with ND-fed mice (Figure 1D, Group C vs A and Group D vs B). ~~Furthermore, increased IL-17A secretion by ELNC correlated with higher activity of MPO in ear tissue of the obese mice (Figure 1E, Group D vs. B).~~ Furthermore, increased IL-17A secretion by ELNC correlated with higher activity of MPO in ear tissue but only in obese mice, which were sensitized and challenged with the hapten (Figure 1E, Group D vs. B). We also found a significant increase in the concentration of TNP-specific IgG1 antibody in the obese mice when compared with the lean controls (Figure 1F, Group D vs. B). TNP specific IgG2c-antibody was also elevated in the obese mice, but the changes were not significant (Figure 1G, Group D vs. B).

3.2 HFD alters the bacterial population toward a proinflammatory profile but HFD-GM transferdoes not affect CHS in the recipients

Studies support the notion of an association between the changes of GM composition and different autoimmune diseases [21] and skin disorders [18]. To determine if the exacerbated CHS response in HFD-fed mice was associated with dysbiosis, we assessed the abundance of some common GM by qPCR after 8 weeks feeding with HFD. Our results showed that some

species of GM were significantly altered in the obese mice compared to lean mice (Figure 2B), whereas others remained unchanged (Figure 2A). Among the tested species, the relative abundance of *Bifidobacterium* spp., *Lactobacillus*, *Clostridium* cluster IV, and *Clostridium coccoides* (cluster XIVa) were comparable between HFD-fed and ND-fed mice (Figure 2A, Group B vs. A), whereas HFD significantly increased the relative abundance of *Enterococcus* spp., segmented filamentous bacteria (SFB) and *Clostridium coccoides – E. rectale* (cluster XIVab) (Figure 2B, Group B vs. A). In contrast, the relative abundance of *Bacteroidetes* was significantly decreased in HFD-fed obese mice compared to the ND-fed mice (Figure 2B far right panel, Group B vs. A). It is known that SFB induce IL-17 mediated inflammation [22] and interestingly the abundance of SFB was significantly increased in HFD-fed mice, which is in line with the increase of IL-17A in ELNC (Figure 1D). Further FMT transfers were performed to test whether a HFD-associated microbiota modulates CHS in lean recipients. The luminal contents of large intestine were harvested from the donors after 8 weeks of feeding with ND or HFD. The fecal supernatant was orally inoculated into the recipient mice, twice a week for two weeks, prior to CHS induction. To test the efficacy of FMT the gut content of recipients were harvested 14 days after first FMT. ~~The results presented in Figure 2C showed the same trends in relative abundance of *Enterococcus* spp., SFB, *Clostridium coccoides – E. rectale* (cluster XIVab) and *Bacteroidetes* were observed in recipients of HFD-modified GM (Group B) but the significant difference was obtained only in *Clostridium* cluster XIVab.~~ The results presented in Figure 2C showed in recipients of HFD modified GM (Group B) the same trends in relative abundance of SFB, Clostridium coccoides – E. rectale (cluster XIVab) and Bacteroidetes but the significant difference was obtained only in Clostridium cluster XIVab. Moreover, 2-week transfer of HFD-modified GM promoted a proinflammatory state in the scAT of the recipient mice with an increase in IL-6 and a decrease in IL-10 concentration (Figure 2D). Our previous study showed that antibiotic-

induced GM dysbiosis downregulated CHS, which could be transferred to naïve recipients, causing suppression of CHS [23]. Interestingly, HFD-induced GM dysbiosis neither transferred exacerbated CHS in the current study (Figure S1A, Group C vs. B), nor did it change the bodyweight of the 2-week FMT HFD recipients (Figure S1B).

3.3 Obesity promotes a proinflammatory profile in T cells and dendritic cells (DCs)

CHS can be transferred into naive syngeneic recipients by *i.v.* injection of T effector cells [24]. To determine if the exacerbated CHS seen in HFDIO mice is transferable, we performed adoptive cell transfer experiments. Naive recipient mice were challenged with TNCB ~~PCl~~ after receiving axillary and inguinal lymph node cells (ALNC) or splenocytes (SPLC) from TNCB~~PCl~~-sensitized ND-fed lean donors or TNCB~~PCl~~-sensitized HFD-fed obese donors. As shown in Figure 3A, the recipients of ALNC and SPLC from obese donors developed increased CHS, compared to the mice that received ALNC or SPLC from lean donors (Group C vs. B and Group E vs. D). Our results demonstrate that HFDIO increased T effector cell function as they transferred aggravated CHS into naive syngeneic recipients. We also found that CHS was significantly less in obese recipients after transfer of CHS-effector cells, isolated from TNCB~~PCl~~-sensitized lean mice, when compared to CHS in lean and obese recipients after transfer of CHS-effector cells from TNCB~~PCl~~-sensitized HFD-fed donors (Figure 3B, Group D vs. C and E respectively). These results suggest that HFDIO created the milieu for induction of more potent T effector cells, but not for the T cells already activated in lean donors.

It is known that CHS responses in mice are mediated by either MHC class II-restricted $CD4^+$Th1 or MHC class I-restricted $CD8^+$Tc1 cells, through locally released IFN-γ and/or IL-17 [25, 26], and these cytokines recruit more inflammatory infiltrates and direct cytotoxic damage to local keratinocytes respectively [27, 28]. To investigate the cellular mechanism

involved in the aggravation of CHS in obese mice, we examined the phenotype of T cells in axillary and inguinal lymph nodes (ALN) and spleen (SPL) after TNCB~~PCl~~-sensitization (induction phase of CHS). We found a significantly higher percentage of $CD4^+IL\text{-}17A^+$ cells in ALN and SPL from HFD-fed mice (Figure 4A and B, far left panel) and increased frequency of $CD4^+CCR7^+$ ALNC (Figure 4A, far right panel). The frequency of $CD4^+IFN\text{-}\gamma^+$ in SPLC, but not in ALNC, was significantly increased in HFDIO mice (Figure 4B, 2nd panel from the left). Further, we found that obese mice had significantly lower frequency of IL-4- and IL-10-producing $CD4^+$ T cells in SPL (Figure 4B, middle panel and 2nd panel from the right), interestingly, but not in ALN (Figure 4A, middle panel and 2nd panel from the right). In contrast, the proportion of $CD4^+CD25^+FoxP3^+$ Treg cells from obese TNCB~~PCl~~-sensitized mice was similar to the TNCB~~PCl~~-treated lean mice (data not shown).

In the initiation phase of CHS, both LCs and dDCs migrate into sLNs and present antigen to naïve T lymphocytes [29, 30]. It is conceivable that the exacerbated CHS response in obese mice was due to the proinflammatory phenotype of DCs. To test this hypothesis, we examined the DCs phenotype in ALN and SPL. We found a higher expression of costimulatory molecules (CD80 and CD86) on $CD11c^+$ DCs in ALN of the sensitized obese mice, compared to the lean controls (Figure 4C, far left panel and 2nd panel from the left). The expression of CD86 in splenic DCs of the obese mice was also significantly increased (Figure 4D, 2nd panel from the left). However, the expression of CD80 in splenic $CD11c^+$ cells was decreased (Figure 4D, far left panel). Interestingly, we found an increased frequency of $CD103^+$ DCs, regardless of CD207 expression, in ALN (Figure 4C, the two panels on the right), whereas the increased $CD103^+$ DCs in the spleen were $CD207^-$ (Figure 4D, far right panel) in TNCB~~PCl~~-immunized obese mice, compared to lean control mice. We also found decreased frequency of $CD207^+CD103^-$ DCs in SPL from TNCB~~PCl~~-immunized obese mice when compared with

lean control mice (Figure 4D, 2nd panel from the right). The gating strategies are presented in Figure S2.

3.4 Proinflammatory milieu in scAT of obese mice

Chen et al. reported that $CD11c^+$ cells from epididymal AT are proinflammatory AT-DCs not macrophages [11]. To investigate the potential role for ATDCs in the enhanced CHS, we analyzed cell infiltrates in the scAT, which is adjacent to the skin. In line with the weight gain shown in Figure 1A, the weight of scAT from obese mice was also significantly increased when compared to the lean controls (Figure 5A, Group C vs. A and Group D vs. B). The proinflammatory cytokine IL-6 was significantly higher in the scAT of both naïve and TNCB~~PCl~~-sensitized obese mice compared to lean controls (Figure 5B, left panel, Group C vs. A and Group D vs. B, respectively). In contrast, the anti-inflammatory cytokine IL-10 was markedly decreased in scAT of the TNCB~~PCl~~-sensitized obese mice, compared to the lean counterparts (Figure 5B, right panel, group D vs. B). Further, we found a significant decrease in the proportion of Treg cells (Figure 5C, left panel, Group D vs. B) but a higher percentage of $CD4^+$ (Figure 5C, middle panel, Group D vs. B) and $CD8^+$ T cells (Figure 5C, right panel, Group D vs. B) in scAT of the obese mice. The expression of CD80 and CD86 (Figure 5D) in $CD11c^+$ cells was also increased in the obese and sensitized mice compared with the lean controls (Group D vs. C). The gating strategies are presented in Figure S3. Our results suggest that obesity promotes an inflammatory milieu in the scAT, which attracts more immune cells and aggravates the inflammation.

3.5 IL-17A is required for the exacerbated CHS in obese mice

Given that IL-17A is the highest up-regulated inflammatory cytokine in our model system (Figure 1D; Figure 4A,B), we hypothesized that IL-17A is essential in aggravated CHS in the HFDIO mice. To test our hypothesis, we fed IL-$17A^{-/-}$B6 mice with HFD for 8 weeks prior to

CHS induction. As presented in Figure 6, IL-17A$^{-/-}$B6 mice fed with HFD gained significantly more weight (Figure 6A, Group B vs. A). In contrast to C57BL/6 mice, obese IL-17A$^{-/-}$B6 mice did not develop enhanced CHS indicated by ear swelling (Figure 6B, Group D vs. B) and histological image (Figure 6C). Moreover, the TNP-specific IgG1 antibody level was not statistically different, although elevated in the obese IL-17A$^{-/-}$B6 mice, compared with the lean IL-17A$^{-/-}$B6 mice (Figure 6D, Group D vs. B in the left panel). There was also no difference in TNP-specific IgG2c antibody level (Figure 6D, Group D vs. B in the right panel).

4. DISCUSSION

Our study has revealed the following new findings: 1) HFDIO leads to exacerbated T cell dependent CHS in C57BL/6 mice; 2) HFDIO promotes a proinflammatory profile of GM and creates the milieu for induction of more potent CHS-effector cells; 3) HFDIO does not modulate the function of effector cells in the CHS effector phase; 4) exacerbated CHS in obese mice is IL-17A dependent. The increased ear swelling correlating with histological changes in obese mice in our study is in line with earlier studies reporting an elevated inflammatory response to haptens and non-specific irritants in obese mice [31]. Recent study published by Rühl-Muth AC et al. showed that HFD increases the initial reaction of mice to TNCB manifesting by higher neutrophil migration and overproduction of IL-6 and TNF- production in the ear tissue. In addition, the increase in saturated fatty acids of the HFD and its control diet created the inflammatory milieu resulting in the induction of CHS independent of TLR2 and TLR4 [32]. Moreover, the human study also reported that obesity exacerbated inflammatory skin diseases, including eczema, atopic dermatitis and psoriasis [33] ~~[32]~~. Interestingly, another human study showed an association of diet-induced weight loss with reduced DTH responsiveness in obese humans [34] ~~[33]~~. Different from our results, Katagiri et al. reported decreased CHS to TNCB ~~PCl~~ in obese C57BL/6 mice [19]. The discrepancy may arise

from 1) the use of a different protocol to study CHS; 2) the use of different sex of mice; 3) the use of HFD with oleic acid-rich oil, which attenuates inflammation in obese mice [35] ~~34~~. It is clear that further investigations and standardization of the experimental protocols, including the mouse usage (strain and sex) are needed.

Obesity is associated with chronic, low-grade inflammation in tissues and promotes the development of numerous diseases. It has been reported that B cells play a pathogenic role in the development of adipose tissue inflammation during obesity, causing increased plasma concentrations of IgG1 antibody [~~35,~~ 36, 37]. The obese mice in our study have increased levels of TNP-specific IgG1 antibody, the isotype associated with aggravated CHS to TNCB ~~PCl~~ [38] ~~37~~. Several studies have indicated the role of IL-17A in the onset and/or progression of chronic inflammatory diseases in obese mice and humans [39-41] ~~38-40~~. Our *in vitro* experiments revealed higher production of IL-17A by ELNC in obese mice, whereas IFN-γ and TNF-α were unchanged when compared with lean mice. Moreover, the higher IL-17A secretion was accompanied by an increased MPO activity in ear homogenates. Interestingly, we found significantly increased IL-17A concentration in ELNC supernatants from naïve HFD fed mice when compared to naïve mice receiving ND. This observation may suggest that HFDIO alone promotes inflammation after hapten application and that IL-17A mediates neutrophils recruitment into the inflammation site [42] ~~41~~. The role of IL-17A in up-regulated CHS during obesity in our study was further confirmed in IL-17A$^{-/-}$B6 mice, which exhibited normal CHS to TNCB ~~PCl~~ despite the presence of obesity.

We found that the stronger CHS in obese mice was transferable to lean recipients by CHS-effector cells from obese donors, whereas CHS-effector cells from lean donors failed to induce stronger CHS in the obese recipients. These results suggest a role for obesity in induction of potent CHS-effector cells, whereas obesity does not affect function of pre-existing CHS-effector cells during the effector phase of CHS. Phenotypic analysis revealed

that effector cells were predominantly proinflammatory $CD4^+$ T cells in the obese mice, including i) increased frequency of $CD4^+$IL-$17A^+$T cells in both ALN and SPL; ii) up-regulated frequency of $CD4^+$IFN-γ^+ in SPL, and iii) lower frequency of $CD4^+$IL-4^+ and $CD4^+$IL-10^+ in SPL. Our data are in agreement with a previous study reporting an increased number of $CD4^+$IL-17^+ cells in the draining lymph nodes of obese mice compared to lean mice whereas the $CD4^+$IFN-γ^+ T cell compartment was unaffected by HFDIO [43] ~~42~~.

During inflammation, dDCs are mobilized rapidly from the skin and home to draining LN within 48 h, preceding the arrival of LCs [44] ~~43~~. We have shown that the enhanced CHS response in TLR- and MyD88-deficient NOD mice is associated with an enriched proportion of Th1-inducing $CD207^-CD103^+$ dDCs and a decreased proportion of tolerizing $CD207^+CD103^-$ LCs in ALN [38] ~~37~~. Our results in the current study showed that the increased CHS in obese mice was accompanied by reduced frequency of $CD207^+CD103^-$ DCs, without affecting splenic Treg cells, although $CD207^+$ dDCs or $CD103^-$ dDCs can induce Treg cells in C57BL/6 mice [45-47] ~~44-46~~. Nagao et al. suggested that LCs and $CD207^+$ dDCs act as negative regulators of Th1 cellular responses since mice lacking both LCs and $CD207^+$ dDCs have an increased number of $CD4^+$IFN-γ^+ SPLC [48] ~~47~~. Our data are in line with these findings, as we found a higher frequency of $CD4^+$IFN-γ^+, along with a lower abundance of $CD207^+CD103^-$ cells in SPL of TNCB~~PCl~~-sensitized obese mice. $CD207^+CD103^+$ and $CD207^-CD103^+$ dDCs also play a role in $CD4^+$ T cell–mediated autoimmune diseases [49] ~~48~~ and we found more abundant populations of $CD207^+CD103^+$ and $CD207^-CD103^+$ DCs in ALN and $CD207^-CD103^+$ DCs in SPL in TNCB~~PCl~~-sensitized obese mice. It is possible that the enhanced CHS response in obese C57BL/6 mice was associated with increased proportions of $CD207^+CD103^+$ and $CD207^-CD103^+$ DCs, which induced subsequently higher abundance of $CD4^+$IL-$17A^+$ and $CD4^+$IFN-γ^+ cells. The higher expression of the costimulatory molecules, CD80 and CD86, in $CD11c^+$ cells in obese mice lends support to this possibility. Taken

355 together, our results suggest that the enhanced CHS response in obese mice is associated with
356 proinflammatory phenotype of DCs and T cells.

357 CCR7 is a key regulator of T lymphocyte migration from the skin to the draining lymph nodes
358 [50] ~~[49]~~. Förster et al. found that impaired migration of T cells to ALN was due to lack of CCR7
359 on T lymphocytes, and the reduction of T cell-mediated CHS reaction [51] ~~[50]~~. A recent study
360 suggests that CCR7 plays a causal role in maintaining innate and adaptive immunity in
361 obesity [37] ~~[36]~~. Our current study demonstrated that obesity resulted in a higher expression of
362 CCR7 on $CD4^+$T cells in local ALN but not in systemic SPL after TNCB ~~PCl~~ sensitization.
363 We also demonstrated a proinflammatory milieu in scAT of TNCB~~PCl~~-sensitized obese mice,
364 suggesting that the immune cell traffic is likely through adipose tissue depots during acute
365 inflammation [52] ~~[51]~~. Our finding supports the notion that AT inflammation occurs in obesity,
366 accompanied by a higher number and activation of proinflammatory cells [10, 53] ~~[52]~~. The
367 proinflammatory state in our study was manifested by 1) increased production of IL-6 but
368 decreased secretion of IL-10; 2) a higher frequency of $CD4^+$ and $CD8^+$ T cells but a lower
369 frequency of $CD4^+FoxP3^+$ cells; 3) elevated expression of costimulatory molecules such as
370 CD80 and CD86 by DCs. Zhong et al. showed that B7 costimulation reduced adipose
371 inflammation by maintaining the number of regulatory T cells in adipose tissue. The authors
372 also demonstrated that decreased B7 expression in obesity appeared to directly impair Treg
373 proliferation and function that led to excessive proinflammatory macrophages and the
374 development of insulin resistant [54] ~~[53]~~. We also found a lower percentage of Treg cells which
375 is in line with the lower expression of CD 80 and CD86 markers in scAT of obese mice
376 (Figure 5B and C). It should be pointed out that in our model, unsensitized obese mice did not
377 show higher expression of CD80 and CD86 by $CD11c^+$ cells as reported previously [11]. The
378 imbalance between a proinflammatory and anti-inflammatory state in scAT in our study

confirmed the presence of obesity-related local and systemic inflammation, which may have enhanced T cell-dependent CHS in the obese mice.

Increasing evidence demonstrates the GM dysbiosis has an impact on immune responses and immune-related disorders [13, 14]. The GM composition can be influenced by several epigenetic factors [55] [~~54~~]. We have previously shown that 2-week oral administration of enrofloxacin disturbs the natural composition of GM and induces regulatory immune cells, which suppress CHS inflammatory response [23]. In this study, we described GM dysbiosis after 8 weeks of HFD feeding in C57BL/6 mice. It has been reported that obese mice and humans have a proinflammatory bacterial community, with a decrease in *Bacteroidetes* and an increase in *Firmicutes* [~~55,~~ 56, 57]. Our results support these findings, as we observed a significantly higher abundance of *Enterococus spp.* (*Firmicutes*, class *Bacilli*) and a decreased abundance of *Bacteroidetes* in the gut of the obese mice. Moreover, it has been reported that *Enterococcus faecalis CECT7121* induces the accumulation of splenic IFN-γ-producing cells in C57BL/6 mice [58] [~~57~~], whereas *Bacteroidetes fragilis* increases the percentage of IL-10–producing $Foxp3^+$ Treg cells in the gut [59] [~~58~~]. Interestingly, in some obese patients, weight loss is associated with significant increase of *Bacteroides-Prevotella* abundance [60] [~~59~~]. Our results indicate a higher presence of *Enterococcus spp* and lower abundance of *Bacteroidetes* in obese mice likely promoted the augmented CHS, accompanied by an increase in $CD4^+IFN\text{-}\gamma^+$, and a decrease in $CD4^+IL\text{-}10^+$ T cells in lymphoid tissue. Importantly, we found a significant increase in relative abundance of SFB in obese mice, which is crucial for Th17 induction [22, ~~60,~~ 61, 62]. The increase of SFB in obese mice in our model indeed correlated with a higher frequency of $CD4^+IL\text{-}17A^+$ cells in ALN and SPL and elevated production of IL-17A by ELNC. GM dysbiosis in obese C57BL/6 was also characterized by changes in three clusters of the genus *Clostridium.* We did not find significant changes in relative abundance of *Clostridium* cluster IV and XIVa, which has been shown to promote Treg cell accumulation [63] [~~62~~]. However,

Clostridium coccoides – Eubacterium rectale (cluster XIVab) was more abundant in the gut of obese mice, supporting the human study demonstrating its reduction in patients in whom weight loss exceeded 4 kg [60] [59]. We previously reported that 2-week FMT from antibiotic-treated donors down regulated CHS in the recipients [25]. Other investigators also showed that 2-week FMT is sufficient to modify the microbiota and immune response in the recipients [64-66] [63-65]. However, in our model 2-week FMT from the obese donors neither affected CHS, nor changed the bodyweight of the lean recipients. Recent data showed that mice housed in SPF conditions are less likely to exhibit phenotypic changes after microbiota transplantation [67] [66]. Thus, we assume that our clean mouse housing conditions can influence the FMT effect. Summarizing, it is possible that 1) the increase in anti-inflammatory SCFA-producing *Clostridium* cluster XIVab (CLXIVab) could be responsible for the lack of apparent influence of the microbiota in the FMT HFD on CHS, 2) 2-week FMT is not sufficient; 3) HFD-induced GM dysbiosis and obesity-related low-grade inflammation need to coexist for the development of aggravated CHS. Thus, HFD is required for the recipients prior to CHS test. It is clear that further investigation is required.

In summary, our study demonstrates that HFD modifies GM composition that promotes a proinflammatory state in scAT. HFDIO also promotes proinflammatory T cells and DCs locally and systemically. Moreover, IL-17A is essential in the pathogenesis of enhanced CHS. Our study provides novel knowledge related to obesity-associated exacerbated CHS and we hope our results will aid in improvement of existing treatment and/or in designing novel treatment for obesity-associated skin disorders.

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FIGURE LEGENDS

FIGURE 1 Enhanced CHS in obese C57BL/6 mice. CHS was induced after 8 weeks feeding with HFD or ND. A, Weight gain in mice fed with ND (group A) and HFD (group B). n= 12/group. B, Ear swelling and ear weight in mice fed with ND (group A and B) or HFD (group C and D). Mice were sensitized (day 0) by application of 5% TNCB ~~PCl~~ to the shaved abdomen (group B and D). Unsensitized mice fed with ND (group A) or HFD (group C) were used as negative controls. CHS and ear weight were measured 24 h after challenge with TNCB ~~PCl~~ (day +4). n= 12/group. C, Histology of ear tissue after challenge with hapten TNCB ~~PCl~~ in unsensitized mice on the ND (group A) and HFD (group C) and in sensitized mice fed with ND (group B) and HFD (group D). Hematoxylin and eosin staining. Scale bar=50 μm. Representative images (magnification: ×40). D, Concentration of IFN-γ, IL-17A and TNF-α in ELNC culture supernatants. Mice were fed with ND (group B) or HFD (group D) and subsequently sensitized and challenged with hapten TNCB ~~PCl~~. Negative control mice

were fed with ND (Group A) or HFD (Group C) and subsequently challenged with hapten. ELNC were isolated 24h after challenge and were cultured with antigen for 48 h. The culture supernatants of ELNC were collected and tested for cytokine concentration using an ELISA. Kit n= 5-7/group. E, MPO activity in ear tissue after challenge with hapten TNCB ~~PCl~~ in unsensitized mice (negative) on the ND (group A) and HFD (group C) and in TNCB~~PCL~~-sensitized mice (positive) fed with ND (group B) and HFD (group D). MPO colorimetric activity assay was used to determine the activity of MPO in samples. n= 6-12/group. F-G, Concentration of anti-TNP IgG1 and IgG2c antibodies in serum after challenge with hapten TNCB ~~PCl~~ in unsensitized mice (negative) on the ND (group A) and HFD (group C) and in TNCB~~PCl~~-sensitized (positive) mice fed with ND (group B) and HFD (group D). The collected sera were tested for antibodies concentration using an ELISA. n= 6-12/group. *P<0.05, **P<0.01, ***P<0.001.

FIGURE 2 HFD modifies GM toward bacteria that induce a proinflammatory profile. Relative abundance of bacterial conserved 16S rDNA fragments in gut contents. qPCR was used to evaluate the alteration of gut bacteria. A, Unchanged relative abundance of *Bifidobacterium* spp., *Lactobacillus*, *Clostridium* cluster IV (CLIV), *Clostridium* cluster XIVa (CLXIVa) in mice fed with HFD vs. ND for 8 weeks. B, Increase in relative abundance of *Enterococcus* spp., SFB, *Clostridium* cluster XIVab (CLXIVab) in mice fed with HFD vs. ND for 8 weeks. Decrease in relative abundance of *Bacteroidetes* in mice fed with HFD vs. ND for 8 weeks. C, Relative abundance of *Enterococcus* spp., SFB, *Clostridium* cluster XIVab (CLXIVab), *Bacteroidetes* in recipients of 2-week FMT HFD vs. FMT ND. D, Concentration of IL-6 and IL-10 in scAT of recipients of 2-week FMT HFD and FMT ND. The culture supernatants of scAT were tested for cytokine concentration using an ELISA. n= 6-8/group. *P<0.05, **P<0.01, ***P<0.001.

669

670 **FIGURE 3** ALNC and SPLC from HFDIO donors transfer elevated CHS into naïve
671 syngeneic recipients. A, Transfer of ALNC (group B) and SPLC (group D) from donors fed
672 with ND and TNCB~~PCl~~-sensitized. Transfer of ALNC (group C) and SPLC (group E) from
673 donors fed with HFD for 8 weeks and TNCB~~PCl~~-sensitized. Number of transferred 4-day
674 immune cells: $8x10^6$ ALNC/recipient and $16x10^6$ SPLC/recipient. Negative control mice did
675 not receive any cells (group A). All mice were challenged with hapten TNCB ~~PCl~~. CHS was
676 measured 24h after challenge with TNCB ~~PCl~~. B, HFDIO does not influence T cell function
677 in the effector phase of CHS. Transfer of ALNC+SPLC from 8-week HFD-fed and
678 TNCB~~PCl~~-sensitized donors into ND-fed recipients (group C) or 8-week HFD-fed recipients
679 (group E). Transfer of ALNC+SPLC from ND-fed and TNCB~~PCl~~-sensitized donors into 8-
680 week HFD-fed recipients (group D). Number of transferred 4-day immune cells: $2x10^7$.
681 Negative control mice did not receive any cells (group A and group B). All mice were
682 challenged with TNCB ~~PCl~~. CHS was measured 24h after challenge with TNCB ~~PCl~~. n=7-
683 16/group. *P<0.05, ***P<0.001.

684

685 **FIGURE 4** HFDIO promotes pro-inflammatory T cells and DCs in obese (group B) vs. lean
686 mice (group A) 4 days after TNCB ~~PCl~~ sensitization. A, The frequency of $CD4^+IL\text{-}17A^+$,
687 $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}10^+$, $CD4^+IL\text{-}4^+$ and $CD4^+CCR7^+$ T cells in the $TCR\beta^+$ population in
688 ALNC. B, The frequency of $CD4^+IL\text{-}17A^+$, $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}10^+$, $CD4^+IL\text{-}4^+$ and
689 $CD4^+CCR7^+$ T cells in the $TCR\beta^+$ population in SPLC. C, The expression of CD80 and
690 CD86, and frequency of $CD207^+CD103^+$ and $CD207^-CD103^+$ cells in the $CD11c^+$ population
691 in ALNC. D, The expression of CD80 and CD86, and frequency of $CD207^+CD103^-$ and
692 $CD207^-CD103^+$ cells in the $CD11c^+$ population in SPLC. Samples were analyzed in a flow

cytometer. Gating information is presented as Supplementary Information. n=5/group. *P<0.05, **P<0.01, ***P<0.001.

FIGURE 5 Mice with HFDIO have a proinflammatory milieu in scAT. Mice were fed with ND (group A and B) or HFD (group C and D). Immunization with hapten TNCB ~~PCl~~ was performed in group B and group D (positive groups). A, Weight of scAT, n=10-14/group. B, Concentration of IL-6 and IL-10 in scAT tissue culture supernatants using an ELISA. n=2-7/group. C, Frequency of $CD4^+FoxP3^+$, $TCR\beta^+CD4^+$, $TCR\beta^+CD8a^+$ T cells. n=2-5/group. D, ~~Expression~~ Freguenacy of $CD80^+$ and $CD86^+$ among $CD11c^+$ cells. n=2-5/group. Samples were analyzed in a flow cytometer. Gating information is presented as Supplementary Information. *P<0.05, **P<0.01, ***P<0.001.

FIGURE 6 Lack of increased CHS in obese IL-$17A^{-/-}$B6 mice. CHS was induced after 8 weeks of feeding with HFD or ND. A, Weight gain in mice fed with ND (group A) and HFD (group B). n= 5-8/group. B, Ear swelling in mice fed with ND (group A and B) or HFD (group C and D). Mice were sensitized by application of 5% TNCB ~~PCl~~ to the shaved abdomen (group B and D). Unsensitized mice fed with HFD (group C) or ND (group A) were used as negative controls. CHS was measured 24h after challenge with TNCB ~~PCl~~. ~~C, Histology of ear tissue after challenge with hapten PCl in unsensitized mice on the ND (group A) and HFD (group C) and in sensitized mice fed with ND (group B) and HFD (group D). Hematoxylin and eosin staining. Scale bar=50 μm. Representative images (magnification: ×40).~~ ~~D~~ C, Concentration of anti-TNP IgG1 and IgG2a antibodies in serum after challenge with hapten TNCB ~~PCl~~ in unsensitized mice (negative) on the ND (group A) and HFD (group C) and in TNCB~~PCl~~-sensitized mice (positive) fed with ND (group B) and HFD (group D).

717 The collected sera were tested for antibodies concentration using an ELISA. n= 2-8/group.

718 ***P<0.001.