Original article

MOLECULAR METABOLISM

Diet-induced obesity promotes myelopoiesis in hematopoietic stem cells

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ABSTRACT

Obesity is associated with an activated macrophage phenotype in multiple tissues that contributes to tissue inflammation and metabolic disease. To evaluate the mechanisms by which obesity potentiates myeloid activation, we evaluated the hypothesis that obesity activates myeloid cell production from bone marrow progenitors to potentiate inflammatory responses in metabolic tissues. High fat diet-induced obesity generated both quantitative increases in myeloid progenitors as well as a potentiation of inflammation in macrophages derived from these progenitors. *In vivo*, hematopoietic stem cells from obese mice demonstrated the sustained capacity to preferentially generate inflammatory CD11c^{+} adipose tissue macrophages after serial bone marrow transplantation. We identified that hematopoietic MyD88 was important for the accumulation of CD11c^{+} adipose tissue macrophage accumulation by regulating the generation of myeloid progenitors from HSCs. These findings demonstrate that obesity and metabolic signals potentiate leukocyte production and that dietary priming of hematopoietic progenitors contributes to adipose tissue inflammation.

Keywords Obesity; Hematopoietic stem cells; Myelopoiesis

1. INTRODUCTION

The prevalence of obesity has tripled in the United States over the past three decades [1]. As a result, the burden of obesity-related morbidity has increased dramatically, generating an urgent need to understand the mechanisms that promote metabolic disease. Substantial evidence supports the contribution of obesity-induced inflammation (metainflammation) to diseases such as diabetes and cardiovascular disease via the pro-inflammatory activation of leukocytes in mice [2–4] and humans [5–7]. In humans and animal models, obesity is associated with a pro-inflammatory activation profile of macrophages in a wide variety of tissues that include the liver, adipose tissue, skeletal muscle, pancreatic islets, and the hypothalamus. A major site of leukocyte activation is visceral adipose tissue where a pro-inflammatory population of CD11c^{+} adipose tissue macrophages (ATMs) accumulates with progressive obesity [8–10]. CD11c^{+} ATMs secrete pro-inflammatory cytokines and promote an M1-like activation profile in adipose tissue that contributes to adipocyte dysfunction and insulin resistance [9,11]. Activated macrophages have also been implicated in metabolic dysfunction in the liver [12], pancreas [13], and central nervous system [14,15]. Numerous studies in mice have shown that suppression of this macrophage activation can uncouple obesity from metabolic disease and the development of insulin resistance [16–18].

The processes that govern the accumulation of pro-inflammatory macrophages in diverse tissues in response to obesity are not fully understood. Obesity-induced ATM accumulation is dependent on the trafficking of monocytes to adipose tissue where they are differentiate into macrophages and surround dead and dying adipocytes [19–23]. Consistent with this, obesity triggers an expansion of the pool of circulating classical blood monocytes (CCR2^{+} Ly6c^{hi}) in mice. Similar observations are seen in obese humans as an increase in the frequency of circulating neutrophils and CD14^{dim} CD16^{+} monocytes is seen in obese insulin resistant patients [24] and is suppressed with weight loss [11,25]. These observations suggest that myeloid cell production and activation may be an important regulated step in generating metainflammation and a biomarker of obesity-induced inflammation. While atherosclerosis prone mice have been shown to harbor a reservoir of Ly6c^{hi} monocytes within the spleen [26], the primary site of monocyte production is the bone marrow (BM) compartment where they are generated from hematopoietic stem cells (HSCs) [27]. HSCs are active participants in peripheral inflammatory responses and can alter their cellular output depending on environmental cues [28]. In sepsis, lipopolysaccharide (LPS) can "push" HSCs to increase production of myeloid cells by direct [29,30] and indirect [31] action on HSCs in order to potentiate peripheral immune responses. Consistent with this, clinical studies have identified an increase in circulating hematopoietic

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progenitors in obese patients suggesting that obesogenic cues can trigger the activation of HSCs [32]. Diabetes associated hyperglycemia has been identified as one potential signal that increases myeloid cell production from the BM [33,34].

Many studies have supported a role for MyD88 and TLR signaling in the development of obesity-associated metainflammation [35]. Activation of these pathways within adipose tissue have been implicated in potentiating HSC activation toward myelopoiesis [36]. These pathways have also been implicated in the activation of myelopoiesis in other inflammatory settings [37]. It remains unclear how TLR/MyD88 on hematopoietic stem cells may contribute to high fat diet-induced inflammation. Based on these observations, we examined the hypothesis that myeloid cell production by HSCs is potentiated by high fat diet induced obesity and plays a crucial role in the generation of pro-inflammatory tissue macrophages. Using mouse models of obesity, we found that obesity induces qualitative and quantitative changes in myeloid BM progenitors that amplify the generation of $CD11c^+$ ATMs. Serial transplantation experiments demonstrate that long term self-renewing HSCs (LT-HSC) retain this property in a cell autonomous fashion. Competitive reconstitution experiments demonstrate that the capacity of HSCs to activate myelopoiesis is dependent on MyD88 for the generation of $CD11c^+$ ATMs in visceral adipose depots.

2. MATERIALS AND METHODS

2.1. Animals and animal care

Mice used in these experiments were male C57Bl/6J, CD45.1 CD57 Bl/6J mice (B6.SJL-*Ptprca Pepcb*/BoyJ), *Tlr4*$^{-/-}$ and *MyD88*$^{-/-}$ (Jackson Laboratories). Heterozygous CD45 1.2 mice were bred for BMT experiments. Mice were fed *ad lib* either a control normal diet (ND) consisting of 4.5% fat (5001; LabDiet) or a high fat diet (HFD) of 60% of calories from fat (Research Diets, Inc., D12492) starting at 6–8 weeks of age for 16 weeks unless specified. In all experiments animals were age matched by purchase or less then one week apart in date of birth. Animals were housed in a specific pathogen-free facility with a 12 h light/12 h dark cycle and given free access to food and water. All animal use was in compliance with the Institute of Laboratory Animal Research Guide for the Care and Use of Laboratory Animals and approved by the University Committee on Use and Care of Animals at the University of Michigan (Animal welfare assurance number A3114-01). Peritoneal macrophages were elicited by 2 ml of 3% thioglycollate injection as previously described [20].

2.2. Cell culture

BM cells were isolated from C57Bl/6 mice by flushing of tibia and fibula. After RBC lysis, cells were plated at 1.5×10^6 cells/ml prior to differentiation into BM derived macrophages (BMMP, 20% L929 conditioned media) or BM derived dendritic cells (BMDC, GM-CSF) for 6 days as previously described [38]. Differentiation was confirmed by demonstrating CD11c and MHCII expression in BMDC by flow cytometry. Cells were then placed in 10% serum media for 24 h prior to treatment with LPS (10–100 ng/ml) for 18–24 h. Cells were sorted by fluorescent activated cell sorting in the University of Michigan core facility for $CD11c^+$ live BMDC or BMMP.

2.3. Microarray and real-time RT-PCR

RNA extraction was performed with an RNeasy kit (Qiagen). RT reactions were performed and real-time PCR analysis was performed normalized to GAPDH (SYBR Green, ABI Prism 7200 Sequence Detection System; Applied Biosystems). Relative expression was assessed by the comparative C_T method correcting for amplification efficiency of the primers and performed in duplicate as previously described [9]. PCR primers used are reported in Supplementary Table 3. For microarray experiments, RNA quality was assessed on Agilent Bioanalyzer Picochip. RNA was amplified and RNA prepared as previously described before hybridization to Mouse Affymetrix 430 2.0 gene strip array [39]. After quality control assessments probe sets with unadjusted *p*-value of 0.05 or less were identified. Genes with significant differences from lean and obese cells were analyzed using DAVID and ConceptGen. Analysis was performed through the University of Michigan Microarray Core using affy, limma, and affy PLM packages of bioconductor implemented in the R statistical environment.

2.4. Adipose tissue stromal vascular fraction (SVF) isolation and flow cytometry

Adipose tissue fractionation and flow cytometry analysis was performed as previously described [40]. Whole adipose tissue was digested in collagenase (1 mg/ml) and SVF fraction pelleted. After red blood cell lysis cells were stained with CD64 PE, CD45.1 PECy7, CD45.2 e450, F4/80-APC, CD11b-APC-Cy7, and CD11c-PE-Cy7 (eBioscience) [9]. Stained cells were analyzed using Canto II cytometer. Initial gating focused on $CD45^+$ singlet cells. Macrophages were identified as $F4/80^+$ $CD11b^+$ or $CD64^+$ cells in the $CD45^+$ gate and then differentiated based on CD11c expression as shown in Supplementary Figure 1.

2.5. Flow cytometry assessment of HSC and myeloid progenitors

BM from one femur was flushed with PBS, made into single cell suspension with a syringe, and centrifuged. Pellet with then treated with RBC lysis solution for 5 min. After re-suspension in PBS and washing cells were stained with lineage markers on APC (CD4, CD5, CD8, CD11b, B220 (CD45R), Gr1, Ter119, CD41) (eBioscience), CD117 APCCy7 (eBioscience), Sca 1 PECy7 (eBioscience), CD150 PE (Biolegend), Endoglin Pacific Blue (Biolegend), CD16/32 PerCP5.5 (eBioscience) and gating as described by Pronk et al. [41]. Initial gating identified lineage (CD4, CD5, CD8, CD11b, B220, Gr1, Ter119, CD41) negative singlet cells. Cells were classified as Lin^- Sca^+ Kit^+ (LSK) or Lin^- Kit^+ (LK). LSK cells were further subdivided between $Endoglin^-$ $CD150^-$ multipotent progenitors (MPP) or $Endoglin^+$ $CD150^+$ long term hematopoietic stem cells (LT-HSC). LK cells were further subdivided between $CD16/32^+$ $CD150^-$ granulocyte macrophage progenitors (GMP) or $CD16/32^-$ $CD150^-Endoglin^-$ pre-granulocyte macrophage (Pre-GM) cells. Representative sample showed in Supplementary Figure 1B. In competitive BMTs CD45.1 and CD45.2 stains were used for determining donors in the blood and SVF. Within the BM compartment and HSC staining CD45.2 staining was used to differentiate the CD45.2 and CD45.1 donor groups.

2.6. Colony forming unit assays

BM from a femur was flushed with IMDM media. Cells were then re-suspended in methocult media and plated at 10,000 cells per plate per protocol (Stem Cell Technology). After 7 days, colonies were counted.

2.7. BM transplantation

BM cells were isolated from donor groups [42] and injected retro-orbitally into lethally irradiated (900 Rad) 6 week old recipient mice (10 million cells/mouse). LSK BMTs were performed with 4000 sorted LSK cells with 0.5×10^6 whole BM from a CD45 1.2 mouse. Competitive BMTs were performed with 5 million cells from each donor mouse (CD45.1 and CD45.2) per recipient. Animals were treated with antibiotics (polymyxin and neomycin) for 4 weeks post BM transplantation. Blood flow analysis was performed to assess reconstitution

