Original article

MOLECULAR METABOLISM

Maternal high-fat diet and obesity compromise fetal hematopoiesis

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ABSTRACT

Objective: Recent evidence indicates that the adult hematopoietic system is susceptible to diet-induced lineage skewing. It is not known whether the developing hematopoietic system is subject to metabolic programming via *in utero* high-fat diet (HFD) exposure, an established mechanism of adult disease in several organ systems. We previously reported substantial losses in offspring liver size with prenatal HFD. As the liver is the main hematopoietic organ in the fetus, we asked whether the developmental expansion of the hematopoietic stem and progenitor cell (HSPC) pool is compromised by prenatal HFD and/or maternal obesity.

Methods: We used quantitative assays, progenitor colony formation, flow cytometry, transplantation, and gene expression assays with a series of dietary manipulations to test the effects of gestational high-fat diet and maternal obesity on the day 14.5 fetal liver hematopoietic system.

Results: Maternal obesity, particularly when paired with gestational HFD, restricts physiological expansion of fetal HSPCs while promoting the opposing cell fate of differentiation. Importantly, these effects are only partially ameliorated by gestational dietary adjustments for obese dams. Competitive transplantation reveals compromised repopulation and myeloid-biased differentiation of HFD-programmed HSPCs to be a niche-dependent defect, apparent in HFD-conditioned male recipients. Fetal HSPC deficiencies coincide with perturbations in genes regulating metabolism, immune and inflammatory processes, and stress response, along with downregulation of genes critical for hematopoietic stem cell self-renewal and activation of pathways regulating cell migration.

Conclusions: Our data reveal a previously unrecognized susceptibility to nutritional and metabolic developmental programming in the fetal HSPC compartment, which is a partially reversible and microenvironment-dependent defect perturbing stem and progenitor cell expansion and hematopoietic lineage commitment.

Keywords Developmental programming; Hematopoietic stem and progenitor cells; Hematopoiesis; High-fat diet; Obesity; Fetal liver

1. INTRODUCTION

The rise in obesity rates over the past several decades coincides with an increased disease burden in obese individuals and their children. Accumulating epidemiologic and experimental evidence strongly suggests maternal obesity and improper prenatal nutrition provide maladaptive intrauterine cues to developing offspring, ultimately programming organs for predisposition to chronic disease later in life [1,2]. Several studies point to developmental origins of postnatal neurological, cardiovascular and endocrine complications via maternal high-fat diet (HFD), a simplified model of the western-style diet, in the absence of gross organ compromise during infancy [3–7]. These multiple lines of evidence present prenatal development as a period of global susceptibility for diet-induced metabolic injury, fetal programming and postnatal organ dysfunction [8], but it remains unclear as to what extent the developing hematopoietic system is vulnerable.

Adverse developmental programming predisposes individuals to chronic conditions, such as metabolic syndrome [9,10], in which inflammation plays a substantial role [7,11], yet no studies have addressed the impact of fetal programming on the hematopoietic stem cells (HSC) from which adaptive and innate immunity arise. HSCs rely heavily on glycolysis and fatty acid oxidation, and the requirement for them to switch between metabolic states for cell fate decisions—quiescence, self-renewal, or differentiation—leaves them susceptible to nutritional perturbations [12]. HSCs first emerge in the early embryo and go on to rapidly expand in the fetal liver before

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transitioning to the bone marrow late during gestation [13]. Here, we expand our previous observations that maternal overnutrition severely stunts liver size [14] by asking if the fetal hematopoietic stem and progenitor cell (HSPC) pool is sensitive to metabolic injury.

We report that HFD and maternal obesity perturb both the expansion of the fetal HSPC pool and hematopoietic lineage specification, using the C57BL/6 mouse model of diet-induced obesity. These studies demonstrate a link between developmental programming and canonical regulation of self-renewal and cell migration, known to constrain HSPC function. Our observations of maternal diet- and obesity-induced vulnerability of the fetal HSPC pool provide novel mechanistic and functional evidence for *in utero* metabolic programming of the hematopoietic system.

2. MATERIALS AND METHODS

2.1. Mice

Animals were handled in accordance with OHSU IACUC. For HFD studies, C57BL/6 CD45.2 female mice (Jackson Labs) were fed a 60% kcal% fat diet (D12492, Research Diets, New Brunswick, NJ) or a 13.5% kcal% fat, control diet (Laboratory Rodent Diet 5001, Lab Diet, St. Louis, MO) *ad libitum*. Acute HFD mice were fed HFD for 2 weeks starting at 9–11 weeks of age and age-matched controls were kept on control diet; both groups were kept on respective diets through breeder pairing and pregnancy. Chronic HFD female mice were fed HFD starting at 5–7 weeks of age, and together with corresponding control diet mice, were sacrificed for fetal harvests at 26–37 weeks of age; HFD-induced obesity but not overt diabetes occurs in these animals. For DR experiments, mice from the chronic HFD cohort were switched to the control diet at 42 weeks of age, bred, kept on control diet through gestation (age-matched controls remained on control diet), and sacrificed at 45–49 weeks of age for fetal harvests. Males were only fed HFD when paired with females for breeding. Pregnancies were timed using the vaginal plug method; fetuses were harvested at day 14.5 of gestation and livers were dissected and prepared in single cell suspensions by pipetting. Cells were counted by hemacytometer or Bio-Rad TC10. Masses were collected as wet weights.

2.2. Cell culture

Unfractionated fetal liver cells were plated in mouse methylcellulose complete media (R&D Systems, Minneapolis, MN) at 20,000 cells/mL and performed according to manufacturer's instructions.

2.3. Flow cytometry

Cells were prepared from fetal liver and adult bone marrow. The following antibodies were used for analysis: CD3, CD4, CD5, B220, Gr-1, Ter119, c-Kit/CD117, Mac-1, CD45.2 (BD, Franklin Lakes, NJ), CD45.1, Sca-1, AA4.1/CD93, F4/80 (eBioscience, San Diego, CA). Staining reagents were also used for analysis: LIVE/DEAD Fixable Dead Cell Stain (Invitrogen, Carlsbad, CA), propidium iodide (Sigma–Aldrich, St. Louis, MO), Annexin V, and the reactive oxygen species dye carboxy-H_2DCFDA (Molecular Probes, Eugene, OR). Cells were analyzed on a BD LSR II and a BD FACS Calibur.

2.4. Quantitative real-time PCR

For qRT-PCR, HFD or control fetal liver cells were immunomagnetically enriched using the Sca-1 antibody conjugated to PE (clone E13-161.7; BD, Franklin Lakes, NJ) and the EasySep PE Positive Selection Kit (StemCell Technologies, Vancouver, BC) [15]. RNA was extracted with an RNeasy Mini Kit (Qiagen, Valencia, CA), cDNA synthesis was performed with SuperScript III First-Strand (Invitrogen), and reactions were run on StepOnePlus (Applied Biosystems, Foster City, CA). Primers were previously described for *Bmi1* [16], *Hmga2* [17], *Igf2bp2*, *Lin28* [18], and *Mmp9* [19], and normalized to β*-actin*. Primer sequences for murine *Mmp8* and *Egr1* are in Appendix Table A.1. Data was analyzed by using the comparative C_T method. Statistics were performed on the relative fold change ($2^{-\Delta\Delta CT}$) of each sample, compared to the mean of the respective control cohort.

2.5. Statistical analysis

Means are presented in bar graphs and scatter plots, $\pm$standard error of the mean (error bars), and compared using two-tailed, unpaired Student's *t*-test; a *P* value of 0.05 or less is considered significant. Where appropriate, the false discovery rate is calculated and the corrected significance values (*q*) are presented [20].

2.6. RNA-sequencing and analysis

A cohort of dams were fed HFD or control diet for 12–14 weeks, and their offspring were harvested at 15 $\pm$ 0.5 dpc; whole livers from 6 control and 6 HFD male fetuses were frozen in RNALater (Qiagen). Total RNA was extracted using RNeasy (Qiagen). An mRNA library was prepared by the OHSU Massively Parallel Sequencing Shared Resource, using the Illumina TruSeq RNA Sample Prep Kit v2. Starting with total RNA, mRNA was purified using polyA selection, then chemically fragmented and converted into single-stranded cDNA using random hexamer priming. Next, the second strand was generated to create double-stranded cDNA. Blunt-end DNA fragments were then generated using fill-in reactions and exonuclease activity. An 'A'-base was added to the blunt ends of each strand, preparing them for ligation to the coded sequencing adapters. Once the adapters were ligated, the constructs were then subject to 10 rounds of PCR. The amplified libraries were purified and underwent quantitative PCR with an ABI StepOne real-time PCR system. Libraries are diluted to an empirically determined concentration appropriate for the flow cell in use and applied using an Illumina cBot. Flow cells were sequenced on an Illumina HiSeq 2000.

We assessed read quality using fastqc (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) metrics and aligned to the mm10 genome using Subread 1.3.6-p1 [21]. The biomaRt package from Bioconductor [22] was used for annotation. EdgeR was used to determine differentially expressed genes [23]. *P*-values were adjusted for multiple testing using the Benjamini–Hochberg method [20]. Differentially expressed genes were further filtered by coefficient of variation (CV), calculated as the standard deviation divided by the mean; a gene passed the CV filter if the CV for both the control group and HFD group was less than 2. Processes of interest in which significantly differentially expressed genes are involved were identified by GO Terms from Bioconductor. The data are accessible in the NCBI Gene Expression Omnibus (GEO) through accession number GSE62715 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62715).

2.7. Transplantation

For non-competitive transplantation, CD45.2 females were placed on HFD or control diet at 7–10 weeks of age, and after 20–23 weeks, were bred for timed pregnancies. On the day of fetal harvest (14.5 dpc), 8–13 week-old male CD45.1 mice were irradiated in a cesium irradiator (J.L. Shepherd) with 750 cGy and approximately 7 h later, were each injected with 1×10^6 whole fetal liver cells. Each recipient received a different fetal liver, for a total of 5 chronic HFD and 5 control grafts. Retroorbital blood draws were performed at 4 and 14 weeks post-transplant and peripheral blood was stained with antibodies against CD45.1 and CD45.2 for analysis.

