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In utero exposure to gestational diabetes mellitus conditions TLR4 and TLR2 activated IL-1beta responses in spleen cells from rat offspring

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ABSTRACT

Fetal exposure to gestational diabetes mellitus (GDM) is associated with a higher risk of youth-onset insulin resistance and type 2 diabetes. We have previously shown that the rat offspring of GDM dams are insulin resistant when compared to the offspring of lean dams. Since inflammation influences insulin sensitivity, we examined the impact of fetal exposure to GDM on inflammatory responses in the offspring. In rats, we compared inflammatory activity in newborn pups as well as 16 week-old young-adult offspring from lean control dams with offspring from high fat and sucrose diet (HFS)-induced GDM dams. To determine whether there are additive effects of exposure to GDM and post-weaning diets, offspring of lean and GDM dams were fed either low fat or HFS diets until 16 weeks of age. Plasma levels of interleukin(IL)-1β were elevated in the offspring of GDM dams. To determine whether this was related to immune reactivity, spleen cells from both the newborn and 16 week-old offspring were isolated and reactivity to the toll-like receptor activators, pam3CSK4 and lipopolysaccharides were measured over a 72 h timeframe. Spleen cells of GDM dams exhibited sustained stimulation of interleukin(IL)-1β and IL-10 production, whereas IL-1β and IL-10 synthesis diminished over time in spleen cells from the offspring of lean dams. Additive effects of GDM exposure and post-weaning HFS diet were not observed, suggesting the effects of GDM on cytokine production are independent of the post-weaning diet. Thus, we conclude that exposure to GDM in utero may condition the immune reactivity of spleen cells.

1. Introduction

Obesity is a worldwide epidemic and rising numbers of children are becoming obese and developing T2D [1,2]. In parallel with this trend, increasing numbers of women are overweight or obese when they become pregnant, coinciding with a steadily rising incidence of gestational diabetes mellitus (GDM) [3]. GDM is characterized by glucose intolerance and hyperglycemia with first onset during pregnancy, as well as chronic low-grade inflammation and elevated circulating free fatty acids (FFA) [4]. While diet and lifestyle are very important contributors to the rise of childhood obesity and T2D, both population health evidence and animal models with controlled pre- and postnatal conditions suggest that in utero exposure to maternal obesity and diabetes during pregnancy increases obesity and insulin resistance in the offspring (reviewed in [5]).

The spleen is the largest lymphoid organ in the body and functions as a reservoir for a large percentage of the body's monocytes that play an important role in host immune function. Diets high in fat induce Interleukin(IL)-1β, IL-6 and TNF-α production by splenocytes [6]. Systemic low-grade inflammation that is associated with obesity is a major contributor to the development of insulin resistance, which is an integral component of T2D development. In the obese state, monocytes/macrophages infiltrate adipose tissue and produce pro-inflammatory cytokines which can disrupt the metabolic-immunologic homeostatic balance and promote insulin resistance [7]. The spleen produces B-cells and T-cells, B-cell and T-cell numbers are increased by obesity; the depletion of these cells improves insulin sensitivity [8–10]. B-cells and T-cells appear to infiltrate adipose tissue prior to macrophages and consequently the production of pro-inflammatory cytokines by B-cells and T-cells contributes to macrophage activation [11,12]. Within this context, toll-like receptor (TLR)4 and TLR2 have been implicated as triggers for inflammation. Typically activated in response to bacterial carbohydrates, TLR4 and TLR2 can

Abbreviations: FFA, free fatty acid; GDM, Gestational Diabetes Mellitus; HFS, High Fat and Sucrose; IL, Interleukin; LF, Low Fat; LPS, Lipopolysaccharide; T2D, type 2 diabetes; TG, triacylglycerol; TLR, toll-like receptor; TNF, tumour necrosis factor.

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also bind dietary fatty acids, such as palmitate, resulting in chronic low-grade inflammation. The activation of TLR4 and TLR2 results in the production of IL-1β and other pro-inflammatory cytokines. IL-1β appears to be a "gatekeeper" of metabolic disease [13] as it has the capacity to inhibit transcription of the insulin receptor, as well as mediate pancreatic β-cell destruction, which affects both insulin resistance and secretion [14,15]. Moreover, inhibition of IL-1β can ameliorate dysglycemia and inflammatory processes in metabolic diseases such as T2D [13,16–19].

Currently, little is known about how GDM exposure conditions the inflammatory responses of the offspring and contributes to their susceptibility to metabolic disease development. To investigate the early life conditioning effects of GDM on the immune reactivity of the offspring, we utilized our high fat and sucrose (HFS) diet-induced GDM rat model that is associated with maternal obesity [20]. GDM dams develop mild mid-gestational hyperglycemia, hyperinsulinemia and glucose intolerance, which reflects the clinical features of GDM [4,20]. Young adult offspring of the GDM dams have been shown to develop obesity and insulin resistance [20]; however, the impact of GDM exposure on immune reactivity of the offspring has not yet been investigated. In the present study, we report that fetal exposure to maternal GDM influenced immune reactivity and increased the production of cytokines in spleen cells derived from the offspring of GDM dams, suggesting that adverse induction of immune reactivity in the offspring may be an important driving force behind the development of T2D and metabolic disorders in GDM-exposed offspring.

2. Materials and methods

2.1. Diet-induced GDM model

All procedures were approved by the Animal Welfare Committee of the University of Manitoba, and adhered to Canadian Council on Animal Care and the Council for International Organizations of Medical Sciences guidelines. The experimental design for the model of the diet-induced GDM model has been previously published, including the metabolic characteristics of the dams and their offspring [20]. Briefly, three week-old female Sprague-Dawley rats (University of Manitoba) were randomly assigned to a low fat (LF) diet (10% fat, Research Diets D12450B) or a HFS diet (45% fat, Research Diets D12451) for 6 weeks and then mated. Female rats were maintained on the same diets throughout pregnancy and the suckling period. At birth, litters were reduced to eight male pups to avoid competition for food. Male offspring from the LF-fed control dams were designated as the "Lean" control group. Male offspring from HFS-fed dams were designated the "GDM" group. For the experiments involving young adult offspring, Lean and GDM offspring were weaned at 4 weeks of age and were randomly assigned to receive either the HFS or LF diet. Offspring remained on specified diets for a total of 12 weeks post-weaning. Rats were given ad libitum access to food and water and housed two per cage. Neonatal pups were euthanized within 24 h of birth by cervical dislocation. Young adult (16 week-old offspring) were anesthetized using an overdose of sodium pentobarbital and blood was collected by cardiac puncture. Half of the spleen was utilized for isolation of cells for cultured cell experiments. The remaining half of the spleen as well as liver tissues were dissected, rinsed in PBS and freeze-clamped in liquid nitrogen and stored at −80 °C for future analyses.

2.2. Determination of biochemical parameters

Upon collection of blood samples, serum was separated by centrifugation (10 min at 4000 rpm, 4 °C) and stored at −80 °C. Circulating concentrations of insulin, IL-1β, leptin and adiponectin were determined using colorimetric ELISA assays (Millipore, St. Charles, MO USA); triacylglycerol (TG) and FFA were determined by colorimetric assay (Wako Chemicals, Richmond, VA USA). All assays were executed according to manufacturer instructions.

2.3. Spleen cell isolation and culture

As described in Kung et al. [21], spleen cells from 16 week-old offspring were isolated by mincing the spleen in a petri dish and then pressing the tissue through a wire mesh screen into complete medium (10% fetal calf serum in RPMI (Life Technologies, Burlington, Ontario, Canada). Neonatal spleens were pooled (3/litter) and disassociated with sterile glass slides into complete medium. In both cases, cells were washed and resuspended. Cell suspension was layered onto 3 ml of Ficoll-paque (Cedarlane Laboratories, Hornby, Ontario, Canada) and centrifuged. Cells were washed, resuspended in complete medium and counted. Cells were cultured at 4 million cells/well in 6-well round bottom plates (Corning Inc., Corning, NY). Cells were incubated with complete media and TLR ligands: lipopolysaccharide (LPS; 200 and 2 ng/ml, Sigma) or Pam3CSK4 (200 ng/ml and 20 ng/ml, Sigma). Duplicate cultures were established for individual 16 week-old pups. Single cultures were established for pooled neonatal spleens due to a lower number of cells. Supernatants were harvested at 24, 48 and 72 h and stored at −80 °C for the detection of IL-1β, IL-6, IL-10 and tumour necrosis factor (TNF)-α production by ELISA. Respective cytokine ELISAs were purchased from R&D Systems (Minneapolis, MN).

2.4. Analysis of mRNA expression

RNA was isolated from spleens and livers using Quiashredder columns and RNeasy kits (Qiagen, Valencia CA). A Protoscript kit (New England Biolabs, Ipswitch MA) was used to synthesize cDNA. Amplification of cDNA was assessed with a QuantiTect SYBR Green PCR kit (Qiagen) as run on an ABI-7500 real-time PCR detection machine (Applied Biosystems, Foster City CA). Gene expression was assessed in duplicate using the $2^{-\Delta\Delta CT}$ method and data was normalized to β-actin expression as the reference gene. All primer sequences were previously validated and reported [22,23].

2.5. Statistical analyses

Data are presented as mean (±) SEM. Comparisons between groups were evaluated as unpaired *t*-test. Differences in measurements performed within a group over time were analyzed by ANOVA repeated measures. Correlations were determined by Pearson test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. In utero exposure to GDM enhanced IL-1β production in neonatal and 16 week-old offspring

To characterize the influence of GDM on the development of inflammation in the offspring, pre-pregnancy obesity was induced by feeding a HFS diet prior to mating [20]. The continued consumption of the HFS diet throughout pregnancy resulted in mid-gestational hyperglycemia that is characteristic of GDM [20]. Pregnant female rats fed a LF diet over the same timeframe were used as the control group, as we previously described [20]. Post-weaning, the offspring were fed either HFS

Table 1
Insulin Resistance and circulating IL-1β levels.

Dams	Lean				GDM			
Pup Diet	LF (n = 3)		HFS (n = 9)		LF (n = 7)		HFS (n = 9)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
HOMA-IR Index	0.747	0.07	1.43	0.23	1.69*	0.20	2.45*#	0.35
IL-1β (pg/ml)	4.8	1.6	16.7	9.8	35.5	7.0	47.0	16.7

Significant differences are indicated between Lean and gestational diabetes mellitus (GDM) offspring as *p < 0.05 and between low fat diet (LF) and high fat and sucrose diet (HFS) are indicated as #p < 0.05 (ANOVA, with Bonferroni post-hoc test).