Cytokine 71 (2015) 405–408

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Short Communication

Abdominal visceral adiposity influences CD4$^+$ T cell cytokine production in pregnancy

Marlies K. Ozias [a,*], Shengqi Li [a,1], Holly R. Hull [a], William M. Brooks [b,c], Margaret G. Petroff [d], Susan E. Carlson [a]

[a] Department of Dietetics and Nutrition, University of Kansas Medical Center, Kansas City, KS, USA
[b] Department of Neurology, University of Kansas Medical Center, Kansas City, KS, USA
[c] Hoglund Brain Imaging Center, University of Kansas Medical Center, Kansas City, KS, USA
[d] Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS, USA

ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 16 September 2014
Accepted 25 September 2014
Available online 6 November 2014

Keywords:
Obesity
Inflammation
Humans
Abdominal fat

ABSTRACT

Women with pre-gravid obesity are at risk for pregnancy complications. While the macrophage response of obese pregnant women categorized by body mass index (BMI) has been documented, the relationship between the peripheral CD4$^+$ T cell cytokine profile and body fat compartments during pregnancy is unknown. In this study, third trimester peripheral CD4$^+$ T cell cytokine profiles were measured in healthy pregnant women [n = 35; pre-pregnancy BMI: 18.5–40]. CD4$^+$ T cells were isolated from peripheral blood mononuclear cells and stimulated to examine their capacity to generate cytokines. Between 1 and 3 weeks postpartum, total body fat was determined by dual-energy X-ray absorptiometry and abdominal subcutaneous and visceral fat masses were determined by magnetic resonance imaging. Pearson's correlation was performed to assess relationships between cytokines and fat mass. Results showed that greater abdominal visceral fat mass was associated with a decrease in stimulated CD4$^+$ T cell cytokine expression. IFN-gamma, TNF-alpha, IL-12p70, IL-10 and IL-17A were inversely related to visceral fat mass. Chemokines CCL3 and IL-8 and growth factors G-CSF and FLT-3L were also inversely correlated. Additionally, total body fat mass was inversely correlated with FGF-2 while abdominal subcutaneous fat mass and BMI were unrelated to any CD4$^+$ T cell cytokine. In conclusion, lower responsiveness of CD4$^+$ T cell cytokines associated with abdominal visceral fat mass is a novel finding late in gestation.

1. Introduction

Women with pregravid obesity are at a greater risk for pregnancy complications [1]. The biological mechanisms responsible for these clinical outcomes are not well characterized, although there is a strong link to the increased inflammation that occurs in obesity [2]. Obese pregnant women categorized by their pre-pregnancy body mass index (BMI) have greater circulating C-reactive protein (CRP) and IL-6 accompanied by both adipose and placental innate inflammation at delivery compared to healthy weight pregnant women [3,4]. Another investigation found differences in circulating CRP, IL-6, and CCL2 associated to excess adiposity in the first and second trimesters which disappeared by the third trimester [5]. While that study did measure adiposity by skinfold thickness, the majority of research on the impact of obesity in pregnancy uses pre-pregnancy BMI to categorize obesity. Abdominal adiposity is more physiologically relevant to obesity-induced health risks. Abdominal subcutaneous and visceral adiposity are associated with the systemic inflammation seen in obesity-induced health risks and have the potential to play a greater role than whole-body adiposity in obesity-related pregnancy complications. For example, greater abdominal subcutaneous fat thickness measured at 18–22 gestational weeks by ultrasound increases the risk of gestational diabetes mellitus, cesarean delivery, and fetal macrosomia [6].

Chronic, low-grade, inflammation occurs in obesity. In addition to higher circulating IL-6, TNF-α [7], and CRP [8], PBMC in obese individuals have elevated production of TNF-α and IFN-γ and decreased IL-10 compared to lean individuals [9]. Additionally, peripheral CD4$^+$ [10,11] and adipose CD3$^+$ [12] T cell populations increase in individuals with an obese BMI. However, the CD4$^+$ T cell subpopulations change depending on the severity of the

* Corresponding author at: Department of Endocrinology, Phoenix Veterans Affairs Healthcare System, 650 E Indian School Road, Phoenix, AZ 85012, USA. Tel.: +1 602 277 5551x2436; fax: +1 602 200 6004.
E-mail address: mkozias@gmail.com (M.K. Ozias).
[1] Current address: Brooklyn Hospital Center, Brooklyn, NY, USA.

http://dx.doi.org/10.1016/j.cyto.2014.09.009
1043-4666/

obesity-induced complications. In diet-induced obese mice, $CD4^+$ T helper (Th) 1 cells initiate obesity-induced insulin resistance [13] and obese individuals with diabetes mellitus have a larger adipose $CD4^+$ Th1 cell population than normoglycemic lean and obese subjects [14]. In contrast, the increase in $CD4^+$ T cell population in metabolically healthy obese individuals comes from circulating Th2 and regulatory T (Treg) cell populations while the Th1 and Th17 cell counts remain similar to those in lean subjects [11]. The characterization of $CD4^+$ T cells in pregnant obese women has not been performed.

T cells are routinely characterized by their secretion of cytokines, chemokines, and growth factors (collectively referred to as cytokines herein). The majority of research on the impact of obesity in pregnancy focuses on macrophage-related inflammation. In addition, obesity categorized by BMI is characterized by increased circulating inflammation and increased $CD4^+$ T cell population [10]. It is not known how the $CD4^+$ T cell cytokine profile changes in pregnant women based on their weight status (BMI) or adipose tissue mass. The intent of this study was to compare weight status and several measures of adipose tissue mass, including abdominal visceral and subcutaneous fat mass with the stimulated $CD4^+$ T cell cytokine profile in women during the 3rd trimester of pregnancy.

2. Methods

2.1. Participants

Women from the Kansas City metropolitan area were enrolled between February 2012 and March 2013 for this study. Women were eligible if they were English-speaking, 18–44 years old with a singleton pregnancy, planning to delivery at the University of Kansas Hospital, and had a pre-pregnancy BMI between 18.5 and 40 kg/m^2 based on self-reported height and weight. Exclusion criteria included diabetes, hypertension, smoking or drug use during pregnancy, chronic medical conditions known to influence inflammation status, or fear of enclosed spaces. The research protocol and informed consent process adhered to the Declaration of Helsinki and were approved by the Institutional Review Boards/Human Subjects Committees at University of Kansas Medical Center.

2.2. Isolation of $CD4^+$ T cells and cytokine analysis

Blood samples were obtained from subjects in the third trimester ($n = 35$; between 35 and 39 weeks gestation). PBMC were isolated from blood collected in heparin-coated tubes (BD Vacutainer, Franklin Lakes, NJ). Blood was layered onto Histopaque 1077 (Sigma–Aldrich, St. Louis, MO) and centrifuged for 30 min at 400*g*. Mononuclear cell fraction was collected and washed with RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL Penicillin/ 100 μg/mL Streptomycin, 2 mM L-glutamine, 100 μM β-mercaptoethanol before cell viability and count were assessed. PMBCs were frozen in 90% FBS/10% DMSO in liquid nitrogen until $CD4^+$ T cell isolation and stimulation could be performed on all samples. Frozen samples were resuscitated by rapidly raising the temperature to 37 °C and gradual aliquots of complete warmed RPMI added to 5 times the original volume to prevent osmotic shock. $CD4^+$ T cells were isolated from PBMC by positive selection (Miltenyi Biotec Inc., Auburn, CA). $CD4^+$ purity was assessed by flow cytometry (LSR II, BD Biosciences, San Jose, CA) using FITC anti-human CD4 antibody (Biolegend, San Diego, CA) and estimated to be 98 ± 1% and 96 ± 1% in fresh and frozen samples, respectively. In duplicate, cells were stimulated with PMA (50 ng/mL) plus A23187 (250 ng/mL) for 72 h. Samples were harvested on ice and supernatants were collected by centrifugation at 18,400*g* for 10 min at 4 °C and stored at −20 °C until analysis. $CD4^+$ T cell cytokine expression was determined by Milliplex® xMAP technology (Invitrogen Corp, Camarillo, CA) according to manufacturer's instructions. Cytokine supernatants were measured on a Luminex 200 (Austin, TX). Samples were analyzed on one plate to eliminate intervariability.

2.3. Body fat measurement

To determine total body fat and abdominal subcutaneous and visceral fat mass, dual X-ray absorptiometry and magnetic resonance imaging (MRI; Siemens Skyra 3T) were performed as previously described [15].

2.4. Statistical analyses

Subjects' descriptive statistics are presented as mean values ± SD (Table 1). Women were recruited for the study in three categories based on their pre-pregnancy weight and height: normal-weight (18.5–24.9), overweight (25–29.9), and obese 30.0–40.0). Differences between the three BMI categories were analyzed with one-way ANOVA. In order to normalize the absolute value of abdominal fat mass to each subject's size, each abdominal compartment of adipose was divided by the amount of total body fat mass. Linear relationships were calculated between body composition and $CD4^+$ T cell cytokines with Pearson correlation. Actual cytokine medians and ranges are found in Supplemental Table 1. Shapiro–Wilks test was used to test normality, with outcomes variables not normally distributed log-transformed prior to all analyses. All data were analyzed with IBM SPSS Statistics 17.0 software (SPSS, Chicago, IL). *P* values presented are two-tailed and significance was set at 0.05.

3. Results and discussion

There were no associations between the BMI categories (healthy weight, overweight, obese) and $CD4^+$ T cell-produced cytokines (data not shown). Out of the thirty-five detectable cytokines measured only FGF-2 was related (inversely) to total body fat mass ($r = -0.467$, $P = 0.009$). We found no relationships between abdominal subcutaneous fat mass and $CD4^+$ T cell cytokines.

In contrast, abdominal visceral fat mass correlated with $CD4^+$ T cell cytokine production. Ten cytokines were inversely related to visceral fat mass and two cytokines (FGF-2 and GM-CSF) trended toward a significant inverse relationship (Table 2). Notably, IL-17A was negatively correlated with increased visceral fat mass ($r = -0.525$) as well as IL-10 ($r = -0.549$) and soluble CD40 ligand ($r = -0.491$). Additionally, IFN-γ ($r = -0.501$), TNF-β ($r = -0.513$), and IL-12p70 ($r = -0.507$) had significant negative associations with visceral fat mass. Inverse relationships were also observed between visceral fat mass and $CD4^+$ T cell expressed chemokine concentrations: CCL3 ($r = -0.599$) and IL-8 ($r = -0.721$). Lastly, two growth factors were inversely related to visceral fat mass: G-CSF ($r = -0.580$ and FLT-3L ($r = -0.506$).

To our knowledge, no one has assessed the nature of peripheral $CD4^+$ T cells in relationship to obesity in pregnancy even though obese individuals have increased $CD4^+$ T cell populations [10,11]. Total body fat mass (with one exception) and abdominal subcutaneous fat mass were not related to any cytokine response. Only abdominal visceral fat mass was associated with peripheral blood $CD4^+$ T cell cytokine production with lower production of cytokines, growth factors and chemokines. Perhaps as important as finding a relationship between abdominal visceral fat mass and $CD4^+$ T cell responses, we did not find a relationship between a commonly used BMI and cytokine production. Our results reinforce