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Short Communication

Circulating progenitor cells are positively associated with cognitive function among overweight/obese children

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ABSTRACT

Recent evidence has indicated that overweight/obese children may experience cognitive and immune dysfunction, but the underlying mechanisms responsible for the association between overweight/obesity, immune dysfunction, and cognition have yet to be established. The present study aimed to identify a novel link between obesity-induced immune system dysregulation and cognition in preadolescent children. A total of 27 male children (age: 8–10 years) were recruited and separated by body mass index (BMI) into healthy weight (HW: 5th–84.9th percentile, n = 16) and overweight/obese (OW: ⩾85th percentile, n = 11) groups. Adiposity was assessed using dual energy X-ray absorptiometry (DXA), and aspects of executive function were assessed using the Woodcock-Johnson III Tests of Cognitive Abilities. Monocyte populations ($CD14^+CD16^-$, $CD14^+CD16^+$) with and without expression of chemokine receptor type 2 (CCR2), and circulating progenitor cells (CPCs: $CD34^+CD45^{dim}$), in peripheral blood were quantified by flow cytometry. CPCs were isolated by flow sorting and cultured for 24 h for collection of conditioned media (CM) that was applied to SH-SY5Y neuroblastomas to examine the paracrine effects of CPCs on neurogenesis. OW had significantly higher quantities of both populations of monocytes ($CD14^+CD16^-$: 57% increase; $CD14^+CD16^+$: 95% increase, both $p < 0.01$), monocytes expressing CCR2 ($CD14^+CD16^-CCR2^+$: 66% increase; $CD14^+CD16^+CCR2^+$: 168% increase, both $p < 0.01$), and CPCs (47% increase, $p < 0.05$) than HW. CPCs were positively correlated with abdominal adiposity in OW, and negatively correlated in HW with a significant difference between correlations ($p < 0.05$). CPC content was positively correlated with executive processes in OW, and negatively correlated in HW with a significant difference in the strength of the correlations between groups ($p < 0.05$ for correlation between OW and HW). Finally, CPC-CM from OW trended to increase neuroblast viability *in vitro* relative to HW (1.79 fold, $p = 0.07$). These novel findings indicate that increased content of CPCs among OW children may play a role in preventing decrements in cognitive function via paracrine mechanisms.

1. Introduction

The nervous and immune systems have a collaborative relationship where cross talk between these two systems is important for their regulation. The influence of the immune system on cognitive functioning and neurogenesis is becoming better established in both pathological and steady-state conditions (Donzis and Tronson, 2014; Marin and Kipnis, 2013). One mechanism by which the immune system has been shown to influence the brain and cognition is via secretion of cytokines. These effects have primarily been evaluated under pathological, pro-inflammatory conditions, and secretion of pro-inflammatory cytokines, such as interleukins, interferons, and members of the tumor necrosis factor family by immune cells caused a decrease in hippocampal specific tasks (Nemni et al., 1992; Rachal Pugh et al., 2001; Tancredi et al., 1990; Valentine et al., 1998). Additionally, conditions associated with immunological stress and inflammation, such as infection, obesity, or genetic models of immune system dysregulation are also associated with decreased performance on executive functioning tasks (i.e., goal directed processes underlying perception, cognition, and action) and negative effects on the brain (Buckman et al., 2014; Cohen et al., 2006; Kipnis et al., 2008; Dinel et al., 2011; André et al., 2014). Recent work from our group has

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demonstrated that obese/overweight (OW) children have decreased performance on tasks of executive function compared to healthy weight (HW) controls Kamijo et al., 2012a,b. Additionally, obese adolescent rodents had decreased cognitive performance which was associated with impaired neurogenesis (Boitard et al., 2012). The role of specific immune cell populations on brain and cognition, and how chronic immune system dysregulation alters the relationship between the nervous and immune systems have not be well established.

Although the central nervous system (CNS) was believed to be impervious to peripheral immune cells, recent evidence has questioned this belief. The migration of circulating immune cells into the CNS is exacerbated following acute inflammatory conditions, such a stroke, and is facilitated by expression of the chemokine receptor type 2 (CCR2) Djukic et al., 2006. These findings suggest that under certain conditions, peripheral immune cells can enter the CNS. Immune cells are derived from hematopoietic stem/progenitor cells (HSPCs) primarily located within the bone marrow (King and Goodell, 2011). HSPCs can also be found in very small quantities in circulation, and these circulating progenitor cells (CPCs) Bellows et al., 2011 have been shown to contribute to tissue repair via secretion of paracrine factors (Palermo et al., 2005). Interestingly, inflammatory conditions, such as acute infection and obesity, increase the content of CPCs and enhance myeloid differentiation of HSPCs (Bellows et al., 2011; Liu et al., 2015; Zaretsky et al., 2014). The role of CPCs in the brain and cognition has not previously been explored in the context of overweight/obese children.

In the present report, we evaluate the relationship between CPCs, monocyte populations, executive function, and neurogenesis in healthy weight (HW) and overweight/obese (OW) children using a combined *in vivo* and *in vitro* approach. OW children have a compromised immune system (Inzaugarat et al., 2014), decreased performance on tests of executive function (Kamijo et al., 2014; Khan et al., 2015), and decreased hippocampal volume (Bauer et al., 2015; Moreno-López et al., 2012). Thus, comparing HW to OW children allowed us to evaluate the link between immune system dysregulation on cognition. We hypothesized that compared to HW children, OW children would have higher quantities of CPCs and monocyte populations expressing CCR2 in peripheral blood compared to HW, and that CPC and monocyte content would be negatively associated with executive function in OW children. Furthermore, we hypothesized that paracrine factors released from hematopoietic cells isolated from OW children would inhibit neurogenesis *in vitro*.

2. Methods

2.1. Participants

Twenty-seven preadolescent (age: 8–10-years; 16 HW and 11 OW) male children who previously underwent cognitive testing as part of a larger study (Hillman et al., 2014) participated in the present study. HW and OW were matched for age, fat free VO_2 max, and general intelligence (IQ) as determined by Woodcock Johnson III Brief Intellectual Ability tests. Participants provided informed written assent with parental/legal guardian consent to participate in the study and were compensated for their time. This project was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign.

2.2. Anthropometric measures, body composition, and fitness

Participants were separated into healthy weight (HW) and overweight/obese (OW) based on body mass index (BMI) with those having a BMI of $\geqslant$85th percentile of their age-matched peers considered OW (Kuczmarski et al., 2000). Whole body and regional soft tissue composition was measured by dual-energy X-ray absorptiometry (DXA) using a Hologic Discovery A bone densitometer (software version 12.7.3; Hologic, Bedford, MA). DXA measures of interest included whole body fat mass (%) and abdominal fat mass (%). Participants completed a maximal exercise test to assess aerobic fitness on a motorized treadmill using a modified Balke protocol (Armstrong, 2006).

2.3. Whole blood analysis

Participants reported to the lab between the hours of 6:00–10:00AM CST after at least an 8-hour fast. Blood was collected from the antecubital vein into EDTA-anticoagulant tubes. Plasma was aliquoted after Ficoll-Paque separation on EDTA-collected blood prior to isolation of peripheral blood mononuclear cells (PBMCs) used for $CD34^+$ isolation by flow sorting. Lineage negative PBMCs were magnetically separated, incubated with PE-conjugated CD34 antibody (1:100 dilution), and sorted using either an iCyt Reflection flow sorter (iCyt; Champaign, IL) or a FACS ARIA II fluorescence activated sorter (BD; Franklin Lakes, NJ). All isolated CPCs from each participant were plated in 250 μL of Serum Free Expansion Media (StemCell Technologies; Vancouver, Canada) for 3 h. The average concentration of plated cells was 25,000 cells/well. Conditioned media was collected and frozen at -80°C until further analysis.

Quantification of CPCs and monocytes with and without CCR2 expression was conducted from whole blood (200 μL/population) collected in EDTA tubes. Samples were incubated with PE conjugated CD34 (1:40; Invitrogen; Grand Island, NY), FITC conjugated CD45 (1:200; Invitrogen; Grand Island, NY), PE conjugated CD14 (1:100; Invitrogen; Grand Island, NY), FITC conjugated CD16 (1:100; Invitrogen; Grand Island, NY), or CCR2 (CD192; 1:100; Biolegend; San Diego, CA). Gating for CPCs was conducted as previously described (Bellows et al., 2011). Flow cytometric analysis was performed within 3 h of blood collection using an Attune Focusing Flow Cytometer (Life Technologies; San Diego, CA).

2.4. Cognitive tasks

A subset of participants completed a subtest of the Woodcock-Johnson III Tests of Executive Processing (WJ III) to assess cognitive performance as described previously (McGrew and Woodcock, 2001) as part of a larger on-going study (Hillman et al., 2014). The WJ III test of Executive Processing is a test of fluid reasoning and decision-making, which involves making logical and novel decisions (Ferrer et al., 2009; Taub and McGrew, 2004) that requires input from a variety of brain regions (Diamond, 2013).

2.5. Neuroblastoma cell culture and conditioned media experiments

SH-SY5Y cells (CRL-2266; ATCC; Manassas, VA) were plated in growth media at 50,000 cells/well for 24 h prior to addition of conditioned media (CM). CM was derived from sorted CPCs as described above. All CM derived from CPCs sorted from healthy weight participants was pooled (HW-CM), while all CM derived from CPCs sorted from overweight/obese participants was pooled (OW-CM). To ensure that differences in CM experiments were not due to different numbers of CPCs contributing to CM, we added serum-free media to the pooled samples to equalize the number of input CPCs to CM between weight classes. In this way, the concentration (plated CPCs/mL of CM) was equal between HW-CM and OW-CM. As such, we could compare differences between groups caused by alterations in the secretome on an individual cell basis rather than a population or cell quantity basis. Neuroblastomas