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Dysregulation of C-X-C motif ligand 10 during aging and association with cognitive performance

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ABSTRACT

Chronic low-grade inflammation during aging (inflammaging) is associated with cognitive decline and neurodegeneration; however, the mechanisms underlying inflammaging are unclear. We studied a population ($n = 361$) of healthy young and old adults from the MyoAge cohort. Peripheral levels of C-X-C motif chemokine ligand 10 (CXCL10) was found to be higher in older adults, compared with young, and negatively associated with working memory performance. This coincided with an age-related reduction in blood DNA methylation at specific CpGs within the *CXCL10* gene promoter. In vitro analysis supported the role of DNA methylation in regulating *CXCL10* transcription. A polymorphism (rs56061981) that altered methylation at one of these CpG sites further associated with working memory performance in 2 independent aging cohorts. Studying prefrontal cortex samples, we found higher CXCL10 protein levels in those with Alzheimer's disease, compared with aged controls. These findings support the association of peripheral inflammation, as demonstrated by CXCL10, in aging and cognitive decline. We reveal age-related epigenetic and genetic factors which contribute to the dysregulation of *CXCL10*.

1. Introduction

Aging is associated with a heightened and prolonged systemic inflammation, termed inflammaging (Baylis et al., 2013; Franceschi and Campisi, 2014). The central nervous system (CNS) and peripheral immune system are inextricably linked and immunoregulatory signals support and shape the immune system (McAfoose and Baune, 2009; Schwartz et al., 2013). Accumulating evidence links chronic inflammation to cognitive decline and the risk of dementia. For example, mice lacking an adaptive immune system have reduced rates of neurogenesis (Ziv et al., 2006) and manifest cognitive deficits (Kipnis et al., 2004; Ziv et al., 2006). Furthermore, through the use of aging heterochronic parabiosis mouse models, excessive circulating proinflammatory cytokines in older organisms have been linked to neuronal insults and impaired spatial learning and memory (Villeda et al., 2011). In humans, older adults with high blood concentrations of proinflammatory cytokines perform worse on certain cognitive assessments (Baune et al., 2008; Trollor et al., 2012; Wilson et al., 2002) and are at an increased risk of dementia (Koyama et al., 2013), compared to those with low concentrations.

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The molecular mechanisms underlying inflammaging, and the cognitive dysfunctions associated with it, are poorly understood. Since heterogeneity in the immune system between individuals are predominantly accountable by nonheritable influences (Brodin et al., 2015), the augmented inflammaging response may be a result of epigenetic disturbances. Epigenetics concerns mechanisms which mediate genetic control without altering the underling DNA sequence. DNA methylation, for example, is an epigenetic mediator of gene repression, and aging has been shown to dysregulate genome-wide DNA methylation marks (Jung and Pfeifer, 2015) and has been linked to neurodegeneration (Lardenoije et al., 2015).

Here, we investigated a panel of 35 plasma cytokines in physically and mentally healthy young and older human adults to identify age-related immune markers associated with specific measures of cognition. This revealed one cytokine, C-X-C motif chemokine ligand 10 (CXCL10), associated with spatial working memory. We further identified epigenetic mechanisms controlling age-related regulation of *CXCL10*, through DNA methylation at specific CpGs in the promoter. We then tested the relationship of a polymorphism, which removes one of these CpGs, with spatial working memory performance. Finally, we investigated CXCL10 within brain samples obtained from deceased individuals with pathological signs of intermediate Alzheimer's disease (AD) and compared them to aged controls.

2. Materials and methods

2.1. Study population

The study population analyzed was a part of the cross-sectional European multi-centre MyoAge cohort (McPhee et al., 2013). Those with full cognitive data and plasma samples available were included in this analysis ($n = 361$). Analysis consisted of young ($n = 135$) and relatively healthy older ($n = 226$) participants (98.9% Caucasian). A detailed description of the exclusion criteria, designed to ensure the selection of healthy participants and to minimize the confounding effect of comorbidity on sarcopenia, has been reported previously (McPhee et al., 2013). Information regarding lifestyle factors (such as education level, smoking status, and excessive alcohol intake) were self-reported. Excessive alcohol was defined for men as >21 units/week and for women >14 units/week. The local medical ethics committees of the respective institutions approved the study, and written informed consent was obtained from all participants.

2.2. Cognitive assessment

Participants completed the Mini−Mental State Examination (Folstein et al., 1975) and Geriatric Depression Scale (Yesavage et al., 1982) questionnaires to screen for cognitive impairment and depression, respectively. Exclusion criteria were set as; an Mini−Mental State Examination score of ≤23 and/or a Geriatric Depression Scale score of ≥5 points. Cognition (spatial working memory, executive functioning, and episodic memory) were assessed using the Cambridge Neuropsychological Test Automated Battery system (Cambridge Cognition Ltd). In addition, a global cognition score was determined as the sum of the 3 individual outcomes to represent a combined performance as utilized previously (Bradburn et al., 2016). Detailed information regarding each test and their output has been published previously (Bradburn et al., 2016). Each cognitive output was standardized by transforming into a *Z*-score based on the young group's average. A positive score therefore will indicate a higher than average performance and *vice versa*.

2.3. Plasma cytokine quantifications

Fasted plasma cytokines were quantified using either cytokine magnetic bead panels (MILLIPLEX MAP, Millipore; Supplementary Table 1) or sandwich ELISA assays (interleukin (IL) -1Ra, IL-6, IL-10, and tumor necrosis factor (TNF) -α; R&D Systems). For the multiplex immunoassays, assays included an overnight incubation at 4 °C and the use of a magnetic plate washer (Bio-Tek ELx405; Bio-Tek). Plates were processed on a Luminex 200 instrument (Luminex), and protein concentrations determined with the xPONENT software (Luminex, v. 3.1.871).

2.4. Plasma biochemical markers

Fasted plasma glucose, triglycerides, total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were all measured using a Daytona biochemical analyzer (Randox, County Antrim).

2.5. Blood cell counts

Lymphocyte and neutrophil counts were determined from whole blood using the KX-21 Automated Hematology Analyzer (Sysmex).

2.6. DNA extraction

Genomic DNA was extracted and purified from whole blood (buffy coat), cell cultures, and brain tissue samples using the Isolate II Blood DNA kit (Bioline), as per the manufacturer's instructions.

2.7. DNA bisulfite pyrosequencing

Genomic DNA (1 μg) was bisulfite treated via the EpiTect Fast Bisulfite Conversion kit (Qiagen). Primers were designed using the PyroMark Assay Design 2.0 (Qiagen) software and sequences are presented in Supplementary Table 2. The *CXCL10* proximal promoter region was amplified through polymerase chain reaction (PCR) using the PyroMark PCR reagents (Qiagen) with the following conditions: 95 °C (15 minutes), [94 °C (30 seconds), 56 °C (30 seconds), 72 °C (30 seconds)] (50 cycles), 72 °C (10 minutes). Bisulfite pyrosequencing was performed on the PyroMark Q24 system (Qiagen) as per the manufacturer's recommendations. A bisulfite conversion control was included in each sequencing assay to confirm complete bisulfite conversion of DNA.

2.8. Cell cultures

HeLa (ATCC) and U937 (ATCC) cells were maintained in Dulbecco Modified Eagle's medium and Roswell Park Memorial Institute medium 1640 media, respectively. Media were supplemented with 10% heat-inactivated FBS (Sigma), 2-mM L-glutamine (Lonza), and 200 U/mL Penicillin-Streptomycin (Lonza). Cells were maintained at 37 °C with 5% carbon dioxide.

2.9. Real-time polymerase chain reaction

Total RNA (2 μg) was extracted from U937 cells using TriSure (Bioline) and reverse transcribed into cDNA using the Tetro cDNA synthesis kit (Bioline) with the use of random hexamers. Primer sequences are found in Supplementary Table 2. Real-time polymerase chain reaction (RT-PCR) was performed on the Stratagene Mx3000P (Agilent) system using SYBR Green chemistry (SensiFAST HI-ROX, Bioline). RT-PCR was performed with the following program; 95 °C (2 minutes), [95 °C (5 seconds), 60 °C (10 seconds), 72