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DNA methylation patterns are related to co-morbidity status and circulating C-reactive protein levels in the nursing home elderly

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ABSTRACT

Elderly residents of long-term care commonly exhibit a number of age-related health deficits, including impaired physical and cognitive function, chronic illnesses, and chronic inflammation. Given our previous data relating the phenotype and function of innate and adaptive leukocytes from the nursing home elderly to chronic conditions and inflammatory biomarkers, we hypothesized that these factors would influence the regulatory programming of immune cells, thereby contributing to immune dysfunction. Since DNA methylation represents both an important regulatory mechanism of cells as well as a biomarker of health and disease, we sought to characterize the methylome of peripheral blood mononuclear cells (PBMCs) from the nursing home elderly (n = 23; 82–98 years old, 70% female), and compare these patterns to pathological factors such as dementia, co-morbidity score and frailty, and immune-related factors such as serum C-reactive protein (CRP) and cytokine levels and varicella-zoster virus (VZV) vaccine responsiveness. We show that the most significant changes in DNA methylation levels occurred in relation to co-morbidity score, including one site, cg07725579 (FDR-adjusted $p < 0.05$; closest gene, SIRBP2), and nine DNA methylation regions (Stouffer's $p < 0.05$). DNA methylation age, although not strongly correlated with chronological age, was positively correlated with serum CRP levels ($p = 0.007$), and negatively correlated with vaccine responsiveness ($p = 0.035$). To our knowledge, this study is one of the first to describe associations of DNA methylation patterns with pathological and immune-related factors in residents of long-term care, and may provide important clues pertaining to immune cell dysfunction near the end of life.

1. Introduction

The transition to long-term care for older adults is usually predicated by a number of factors that tend to accumulate over the trajectory of aging. These include chronic conditions such as dementia (Rist et al., 2016) and diabetes (Rodríguez-Sánchez et al., 2017), functional impairment (Rist et al., 2016; Rodríguez-Sánchez et al., 2017) and related acute injuries (Salminen et al., 2017), and previous hospitalization (Nuutinen et al., 2017). This undoubtedly contributes to biological perturbations that are also commonly observed with age, including alterations in the function and phenotype of circulating immune cells (Montgomery and Shaw, 2015; Boyd et al., 2013), and an increase in molecules commonly associated with the induction of inflammation (Franceschi and Bonafè, 2003). We have shown that as compared to community-dwelling seniors, the nursing home elderly exhibit an increased proportion of intermediate ($CD14^{++}CD16^{+}$) monocytes and reduced monocyte CX3CR1 cell-surface expression (Verschoor et al., 2014), an increased frequency of myeloid derived suppressor cells (MDSCs) (Verschoor et al., 2013), and an increased frequency of activated neutrophils, which is positively correlated to the levels of circulating tumour necrosis factor (TNF) (Verschoor et al., 2015). Interestingly, those elderly participants with increased intermediate monocytes, MDSCs or activated neutrophils are more likely to have chronic conditions such as cancer, diabetes, and dementia (Verschoor et al., 2014; Verschoor et al., 2013; Verschoor et al., 2015). We have also shown that the nursing home elderly exhibit significantly greater circulating levels of the inflammatory-related acute-phase protein C-reactive protein (CRP) as compared to community-dwelling seniors, which is inversely correlated with the responsive to the varicella-zoster virus (VZV) vaccine (manuscript submitted). As a whole, these data indicate that elderly residents of long-term care exhibit alterations to their immune cell compartment beyond what is expected across the

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trajectory of aging, and that pathological (e.g. chronic conditions) and immune-related factors (e.g. levels of inflammatory biomarkers) are important determinants to this end. Unfortunately, little is known regarding the impact of said factors on the regulatory mechanisms of the cells in this compartment.

DNA methylation is the covalent attachment of a methyl group to the 5′ carbon of a cytosine nucleotide, usually followed by a guanine nucleotide (referred to as a CpG), and is an important epigenetic regulatory mechanism of mammalian cells. It is integral in the determination of myeloid (Álvarez-Errico et al., 2015) and lymphoid (Muegge et al., 2003) cell lineage, as well as gene expression in response to pathogen-associated molecular patterns (Matt et al., 2016), cytokine signalling (Acharyya et al., 2010), and environmental stress (e.g. hypoxia (Alivand et al., 2016)). Interestingly, DNA methylation at specific genomic loci have been observed to change with age (Farré et al., 2015; Lam et al., 2012), doing so in manner that may not be consistent among individuals (i.e. epigenetic drift) (McEwen et al., 2017), or occurs the exact same way regardless of the individual, although may vary by rate (i.e. epigenetic clock) (Horvath, 2013). Recent studies have shown that the DNA methylation patterns of immune cells, normally heterogeneous populations in whole blood or fractionated peripheral blood mononuclear cells (PBMCs), also change depending on the pathological or immune-related factor being examined; for example, obesity (Mendelson et al., 2017), circulating CRP (Ligthart et al., 2016) and all-cause mortality (Zhang et al., 2017). Accelerated epigenetic aging, or one's age estimated using DNA methylation information being greater than their chronological age (Horvath, 2013), has been specifically associated with frailty (Breitling et al., 2016), coronary heart disease (Horvath et al., 2016) and disease-specific (Perna et al., 2016) or all-cause mortality (Chen et al., 2016).

In the following study, we sought to examine the relationship between pathological and immune-related factors commonly altered in residents of long-term care. Specifically, we investigated dementia, co-morbidity status and frailty, and the levels of circulating CRP and cytokines and VZV vaccine responsiveness, respectively. At the level of individual or regional CpGs, DNA methylation patterns were significantly associated with co-morbidity status, while accelerated epigenetic aging was associated with increasing CRP levels and decreasing vaccine responsiveness. Given the relationship between DNA methylation and cellular regulation, our findings will do much for our understanding of the consequences of pathological and immune-related factors on immune cell dysregulation in the nursing home elderly.

2. Methods

2.1. Participants and pathological factors

Participants (n = 23) in the current study were selected from a previously published cohort recruited to investigate vaccine responsiveness in older adults (Lelic et al., 2016). They were recruited from nursing homes in Hamilton and Toronto, Ontario, were between 82 and 98 years of age and 70% were female (Table 1); diseases listed in Table 1 represent those with a prevalence > 10% in the original cohort. Co-morbidity status was measured using a co-morbidity score, similar to the Charlson co-morbidity index (Charlson et al., 1987), while frailty was measured using an index similar to Rockwood's approach (Searle et al., 2008); both are previously described (Lelic et al., 2016). Written informed consent was obtained from all participants or their legally appointed guardian. The study protocol and consent procedures were approved by the McMaster Research Ethics Board and by the nursing homes. Whole blood was collected in heparin-coated and uncoated (serum) vacutainer tubes (BD Biosciences, ON, CA) and was processed within 8 h of blood draw. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by Ficoll-density centrifugation and cryopreserved at − 140 °C in human AB serum (Lonza, Basel, CH) and 10% DMSO. Serum was allowed to clot for 30 mins, centrifuged at 1500 rpm for 10 mins, and cryopreserved at − 80 °C.

Table 1
Description of participants in the current study.

	Nursing home elderly (n = 23)
Age	89 (82–98)
Sex	Male, 7; Female, 16
Diseases*	
Heart failure	10%
Chronic pulmonary disease	10%
Diabetes	13%
Dementia	56%
Peripheral vascular disease	83%
Co-morbidity score	2 (1–3)
Frailty	0.29 (0.24–0.31)
Serum molecules	
CRP (μg/ml)	7.7 (1.9–185)
TNF (pg/ml)	8.9 (6.6–9.5)
IL-1β (pg/ml)	0.34 (0.28–0.84)
IL-6 (pg/ml)	2.4 (1.5–4.5)
IL-10 (pg/ml)	2.2 (1.4–4.8)
Log_2 VZV vaccine response	0.66 (0.15–1.4)

Age presented as mean (min-max), diseases presented as % prevalence, and all other variables presented as median (25th–75th percentile).

* Only dementia was tested against DNA methylation patterns as it is the only disease to have a prevalence between 30 and 70%.

2.2. Immune-related factors: serum cytokines and CRP, and vaccine responsiveness

Serum cytokines TNF, interleukin (IL)-6, IL-1β and IL-10 were measured using the Milliplex MAP Human High Sensitivity T Cell kit (Millipore, ON, CA) according to manufacturer's recommendations. C-reactive protein was measured in serum (1/500 diluted) by sandwich ELISA using the monoclonal capture and detection antibody clones C5 (#ab8279, 1 μg/ml) and C6 (#ab24462, 1 μg/ml) (Abcam, ON CA), respectively, and using native, purified CRP as a standard (Aviva Systems Biology Corp., CA, USA). Log transformation of CRP and cytokines was deemed necessary to minimize the influence of extreme values.

The varicella-zoster virus (VZV) vaccine response was measured by interferon gamma ELISPOT at baseline, and 6-weeks follow-up, as previously described (Lelic et al., 2016). It is presented as the log_2 fold-change response, which is the ratio of the follow-up to the baseline measurement.

2.3. DNA methylation analysis

Genomic DNA for methylation analysis was extracted from cryopreserved PBMCs using a Qiacube automated workstation and the DNeasy blood and tissue kit (Qiagen, CA, USA). Genomic DNA was bisulfite converted using the EZ DNA Methylation kit (Zymo, CA, USA) and subsequently hybridized to the Infinium HumanMethylation450 BeadChip (Illumina, CA, USA) at the Genetic and Molecular Epidemiology Laboratory (Hamilton, ON, CA). Unless specified otherwise, all processing and analysis procedures were performed using the R package 'minfi' (Aryee et al., 2014). Prior to raw data processing, the following probes were removed: those designed to interrogate single nucleotide polymorphisms (n = 65); those located on sex chromosomes (n = 11,648); those targeted to polymorphic CpGs (n = 20,696) and cross-hybridizing probes (n = 40,590) (Triche et al., 2013); and probes with low detection (detection p-value > 0.01 on more than five arrays; n = 274). The final set included 415,055 probes. Following probe filtering, background correction and dye-bias equalization on raw data was performed using Noob (Triche et al., 2013) and normalized using SWAN (Maksimovic et al., 2012). Slide effects were corrected using ComBat (Leek et al., 2012). The frequency of major PBMC subsets

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