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The role of DNA methylation and hydroxymethylation in immunosenescence

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ABSTRACT

A healthy functioning immune system is critical to stave off infectious diseases, but as humans and other organisms age, their immune systems decline. As a result, diseases that were readily thwarted in early life pose nontrivial harm and can even be deadly in late life. Immunosenescence is defined as the general deterioration of the immune system with age, and it is characterized by functional changes in hematopoietic stem cells (HSCs) and specific blood cell types as well as changes in levels of numerous factors, particularly those involved in inflammation. Potential mechanisms underlying immunosenescence include epigenetic changes such as changes in DNA methylation (DNAm) and DNA hydroxymethylation (DNAhm) that occur with age. The purpose of this review is to describe what is currently known about the relationship between immunosenescence and the age-related changes to DNAm and DNAhm, and to discuss experimental approaches best suited to fill gaps in our understanding.

1. Introduction

Age-related DNAm and DNAhm may contribute to immunosenescence by regulating or mediating the regulation of levels of immune-related factors and proportions of immune cell types throughout life. Investigating the role of these epigenetic modifications in immunosenescence may help answer fundamental questions about aging while simultaneously providing valuable information to the field of medicine. The well-established associations of DNAm with age and immune cell types in whole blood support a possible role of DNAm in immunosenescence. DNAhm has not been as well studied in this context, so its potential involvement in aging and immunosenescence represents an open area where a gap in knowledge can be closed. The goal of this review is to discuss current evidence on the possible roles of DNAm and DNAhm in immunosenescence and highlight gaps in our understanding.

In mammalian DNA, methylation is the process whereby an enzyme known as DNA methyltransferase binds a methyl group to a cytosine nucleotide at a CpG site (a cytosine that is directly followed by a guanine from 5' to 3') forming 5-methylcytosine (5mC) (Okano et al., 1999). Common DNA methyltransferases are DNMT3A and DNMT3B, responsible for *de novo* methylation, and DNMT1, a maintenance methyltransferase that preserves the methylation state across mitotic divisions. In addition to methyl groups, hydroxymethyl groups have also been observed to be bound to cytosine nucleotides, forming 5-hydroxymethylcytosine (5hmC) (Fig. 1). Ten-eleven translocation (TET) enzymes are a group of three proteins (TET1, TET2, TET3), each of which is capable of catalyzing 5mC to 5hmC (Ito et al., 2010).

DNAm robustly associates with age, numerous chronic diseases, and has a well-studied role in gene regulation. DNAm signatures across thousands of sites can be used to characterize cell type composition (Houseman et al., 2012; Reinius et al., 2012), and have been shown to mark cell lineage skewing (Li et al., 2014), which is a well-known feature of immunosenescence. Shifts in lineage commitment within hematopoietic stem cells (HSCs), the precursor lineage to major immune cell types (Fig. 2), are a well-documented change that occurs with age (Geiger et al., 2013). Within HSCs, differential DNAm with age has been observed in genes expressed in cell lineages downstream of HSCs (Beerman et al., 2013). T cells, a lineage downstream to HSCs, also undergo age-related shifts in subpopulations (Tu and Rao, 2016). In T

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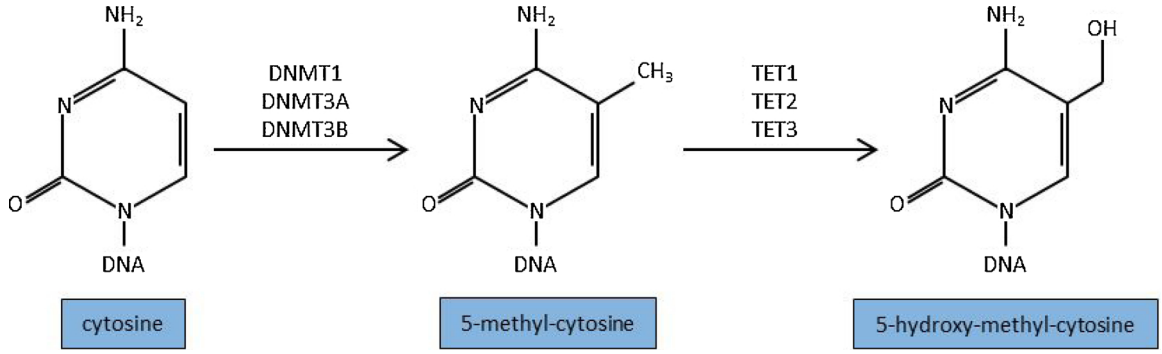

Fig. 1. DNA methyltransferases (DNMT1, DNMT3A, DNMT3B) catalyze the methylation of cytosine to 5-methylcytosine (left and center), and the TET proteins catalyze the hydroxymethylation of 5-methylcytosine to 5-hydroxymethylcytosine.

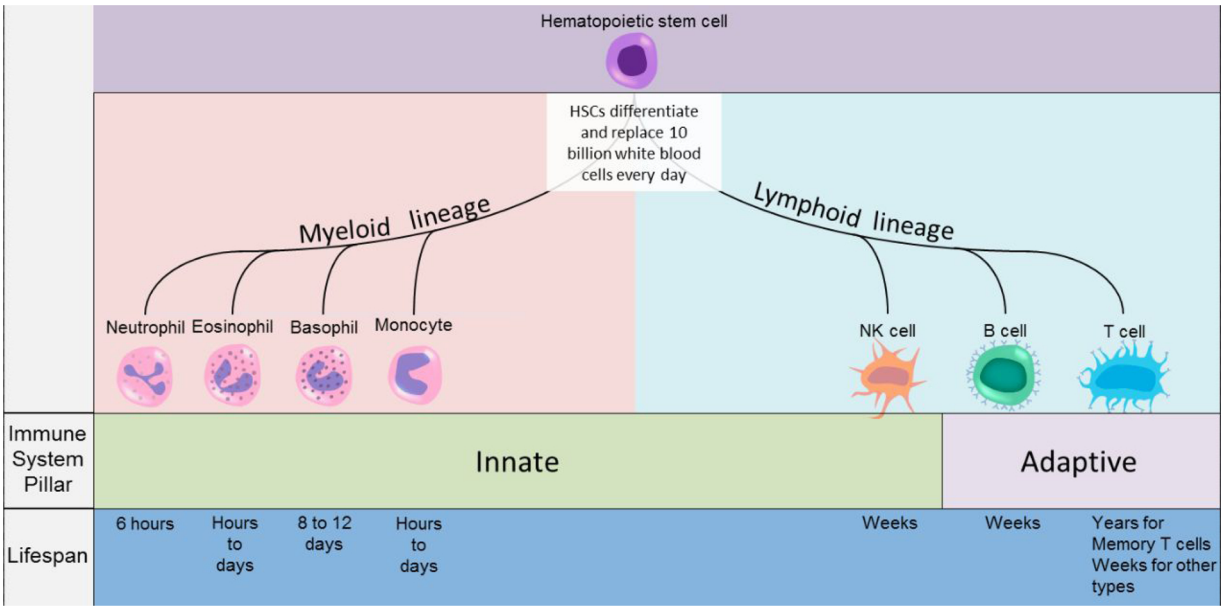

Fig. 2. Lineage differentiation of hematopoietic stem cells into cell types of the myeloid and lymphoid lineage.

cells as well as peripheral leukocytes, association between age-related DNAm and gene expression of genes regulating T cell lineage has also been observed (Tserel et al., 2015). These studies, among others we will describe in this review, support the interpretation that 5mC is involved in immunosenescence.

An appreciation of the possible involvement of 5hmC in immunosenescence has lagged behind that of 5mC. One reason for this is that blood cells carry out numerous functions of the immune system, but 5hmC content is estimated to be 0.027% in human whole blood, which is relatively low (Godderis et al., 2015). 5hmC content in the brain, for example, has been estimated at 13% (Wen et al., 2014). However, the low overall level of 5hmC in blood does not preclude it from playing a regulatory role at specific sites. A second reason is that the discovery of 5hmC in mammalian DNA is more recent and bisulfite-based methods used to detect DNAm are unable to distinguish between 5mC and 5hmC. More recently, however, methods have been developed that use chemical modification to differentiate 5mC from 5hmC prior to sequencing, allowing the specific detection and quantification of 5hmC (Booth et al., 2012; Höbartner, 2011; Song et al., 2012; Szwagierczak et al., 2010; Terragni et al., 2012).

Current evidence suggests that 5mC and 5hmC have distinct effects on gene expression. It is well understood that DNAm can hold a CpG island promoter in a stably repressed state (Illingworth and Bird, 2009; Jones, 2012). In contrast, recent work suggests that promoter DNAhm does not inhibit gene expression like DNAm and instead both promoter and gene body DNAhm associate with increased cis-gene expression (Colquitt et al., 2013; Marco et al., 2016; Zhao et al., 2017a). This may be because proteins (MBD1, MBD2 and MBD4) known to bind to 5mC and contribute to transcriptional repression do not bind to 5hmC (Fig. 3) (Boyes and Bird, 1991; Jin et al., 2010; Nan et al., 1998; Wade, 2001). Instead, 5hmC has been suggested as an intermediate modification between DNAm and demethylation (He et al., 2011; Ito et al., 2011; Tahiliani et al., 2009; Wu and Zhang, 2010). Because they appear to have different regulatory functions, both 5mC and 5hmC warrant consideration when investigating gene regulatory mechanisms in immunosenescence and other biological processes.

The goal of this review is to explore a possible role for DNAm, DNAhm, and gene expression in immune system decline. We start by describing major features of immunosenescence, which is a difficult phenotype to characterize because the immune system is very complex, and the senescence of the immune system involves changes in the quantities of numerous factors carrying out multiple tasks within a variety of blood cell types and their precursor cell lineage, HSCs. We then describe the relationship between epigenetics and the immune system and discuss evidence suggesting that DNAm and DNAhm may play a role in immunosenescence. Next we discuss numerous studies reporting changes in DNAm and DNAhm with age and age-related chronic diseases, which, in many cases occur within immune-related genes or associate with their expression. In some cases, findings suggest some of these immune-related genes to play a role in the degeneration

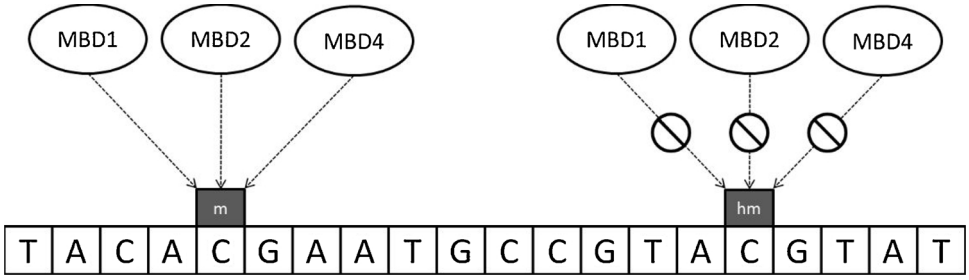

Fig. 3. Methyl-binding proteins (MBD1, MBD2, MBD4), which repress transcription, can bind to methyl groups (m), but not hydroxymethyl groups (hm).

or deterioration characteristic of the disease being interrogated, but in most cases associations are observed without evidence of causality being established, leaving a gap in knowledge to be filled by future studies. We end by outlining potential future directions that could address this gap and advance the field.

2. Immunosenescence

Immunosenescence entails changes in both the innate and adaptive arms of the immune system with age, and is accompanied by immune lineage skewing and an increase in chronic, low-grade inflammation known as inflammaging (Agarwal and Busse, 2010; Franceschi et al., 2000). The cells of the innate and adaptive immune system differentiate from HSCs, which help replace roughly ten billion white blood cells every day of a person's life (Yoder, 2004). Leukocytes (white blood cells) in the human body originate from the HSCs in the bone marrow that first differentiate into the myeloid and lymphoid lineages, thereafter differentiating into neutrophils, eosinophils, basophils, monocytes, and lymphocytes (Fig. 3). Monocytes have the potential to differentiate into macrophages or dendritic cells (DCs) and populate tissues (Geissmann et al., 2010). The lymphocytes of the lymphoid lineage then differentiate into natural killer (NK) cells, T cells, and B cells (Ye and Graf, 2007). Collectively, these cell types carry out various tasks of the immune system, such as phagocytosis, the recognition of "non-self" antigens, and antigen presentation (Janeway, 2001; Medzhitov et al., 2002). In this section we first discuss major age-related changes to immune cell types followed by a discussion of the possible role these changes play to the health and aging of the organism.

2.1. Age-related changes in immune cell types

Innate immune system cells function as first responders to bacterial invaders or other microorganisms. These cells can phagocytose invaders and release cytokines and chemokines alerting other innate immune system cells of the invaders. The release of cytokines and chemokines, also known as the inflammatory response, alerts cells of the adaptive immune system, which are capable of "remembering" specific pathogens and targeted responses to these pathogens in the event of future invasions (Janeway, 2001). Cell lineages of both the innate and adaptive immune system are observed to undergo changes in immunosenescent individuals. HSCs, from which these cell lineages derive (Fig. 3), also undergo changes with age, including reduced function and skewing toward the myeloid lineage (Geiger et al., 2013).

2.1.1. Adaptive arm of the immune system

T cells and B cells are major cell types of the adaptive immune system and work in a coordinated fashion. B cells are responsible for producing antibodies, which respond to specific antigens allowing the adaptive immune system to mount targeted responses to pathogens. T cells carry out many tasks of the adaptive immune system They provide immunity to intracellular and extracellular pathogens, mount attacks in response to infections, and retain "memory" of previous infections so that they can mount attacks in the event of future infections (Zhang and Bevan, 2011; Zhu and Paul, 2008).

One of the most prominent features of T cell aging relates to thymic involution (shrinkage of the thymus with age). Palmer (2013) reviews several studies indicating that thymic involution is responsible for a decreased output of naive T cells in animal models whereas in humans it remains a topic of debate. Regardless, naive T cell output has been observed to decrease with age in humans. In PBMCs of 39 human donors aged 6–90 years, T cell receptor diversity decreased with age, accompanied by a decrease in naive T cells (Britanova et al., 2014). Further, $CD8^+$ T cells are increasingly absent of CD28 with age (Weng et al., 2009).

Many age-associated changes in transcription are observed in T cells and their subsets (i.e. $CD4^+$, $CD8^+$, $CD8^+$, $CD28^-$) (Chen et al., 2013). In $CD8^+$ T cells isolated from PBMCs of five young (23–27 years) and four old (65–80 years) individuals, Cao et al. (2010) observed 754 differentially expressed genes, 66% with decreasing expression and 34% with increasing expression, with overrepresentation of genes involved in immune response among genes with increasing expression. In $CD4^+$ T cells isolated from PBMCs of 423 participants in the Multi-Ethnic Study of Atherosclerosis (MESA), differential expression with age was observed in 218 genes (Reynolds et al., 2015), with suggestive enrichment for immune response pathways among genes with increasing expression with age. In human PBMCs, T_h1 and T_h2 cell counts increase with age while the ratio of T_h2 to T_h1 cells decreases with age (Uciechowski et al., 2008).

B cells, which are responsible for producing antibodies, also undergo functional changes with age, which are reviewed by Cancro et al. (2009). B cells of aged individuals have reduced protein levels and expression of genes that contribute to developmental progression of B cells. Accompanying these changes, B cells have a diminished capacity to complete each stage of differentiation. Age-related alterations in the B1 and B2 cell subsets may reflect these changes: the B1 pathway predominates in prenatal and neonatal development whereas the B2 pathway predominates in young adult life, followed by a proportional increase in the B1 pathway in later life. In addition, B cells undergo a loss in receptor diversity with age (Cancro et al., 2009).

2.1.2. Innate arm of the immune system

Cell lineages, including monocytes, macrophages, and dendritic cells (DCs) of the innate arm of the immune system undergo changes with age as well. Monocytes carry out various tasks in the immune system. They are the most numerous mononuclear phagocyte in the blood, and are capable of migrating from blood to tissues during inflammation, differentiating into macrophages, presenting antigens to T cells, and affecting T cell differentiation (Jakubzick et al., 2017). Despite their similarities, DCs and macrophages have distinct functions: while macrophages are primarily engaged in maintenance of tissue immune integrity, such as bone homeostasis, DCs are efficient antigen presenters primarily involved in tissue immune response (Hashimoto et al., 2011).

In monocyte samples of 146 healthy adults (20–84 years), phagocytosis of monocytes was impaired with age and they exhibited altered

expression of a number of CD molecules (Hearps et al., 2012). In 181 healthy adult subjects (18–88 years), the $CD14^+CD16^+$ subset of monocytes increased with age, accompanied by age-related changes in chemokine receptors and increases in serum monocyte chemoattractant protein-1, although total monocyte counts were comparable between young and old groups (Seidler et al., 2010). In $CD14^+$ monocytes isolated from PBMCs of 1,264 MESA participants (55–94 years), differential expression with age was observed in 2,704 genes, with nominally significant enrichment for immune response pathways (Reynolds et al., 2015).

While monocytes differentiate into macrophages, a recent review notes that the majority of macrophages derive from embryonic precursors and are self-maintained in tissues (Ginhoux and Jung, 2014). In a review of macrophage function and age-related functional decline, Linehan and Fitzgerald (2015) report a number of studies indicating that macrophages show age-related impairments in their ability to become activated in response to IFN-γ, to secrete cytokines in response to TLR stimulation, to present antigens, and to phagocytose and repair tissue. Agarwal and Busse (2010) review a number of murine and human studies suggesting mixed support for altered secretion of chemokines and other cytokines with age in macrophages and monocytes, including reduced levels of TNF-α, IL-1β, IL-2, IL-6, IL-12, CCL5, macrophage inflammatory protein 1α, and increased levels of IL-10. They note inconsistent findings that could be attributable to the fact that many of the murine studies were conducted using stimulated spleen cell cultures of macrophages whereas human studies were carried out using monocytes isolated from peripheral blood (Agarwal and Busse, 2010).

With age DCs have impaired antigen processing and migration, but comparable cell count and phenotypes (Agrawal and Gupta, 2011). In addition, Wong and Goldstein (2013) review a number of studies that collectively suggest an age-related impaired ability of DCs to present antigens to T cells, although some conflicting reports exist. Gupta (2014) reviews mechanisms underlying age-related functional decline of dendritic cells (DCs), noting impaired phagocytosis and migration, and possible consequences of these age-related changes to other immune cell types.

Neutrophils and eosinophils, which are immune cells critical for combating microorganisms and parasites, respectively, also undergo age-related changes. Lord et al. (2001) describe a number of changes in neutrophils including a reduction in the number of microbes ingested per neutrophil, decreased Fc-mediated phagocytosis, and decreased expression of CD16 with age. They suggest low GM-CSF (due to the shift from T_h1 to T_h2 in T cells) and high TNF-α (due to increased secretion in monocytes) may be responsible for age-related reduction in neutrophil responsiveness and decreased survival at the site of infection (Lord et al., 2001). Eosinophils secrete granules containing antimicrobial substances (Faurschou and Borregaard, 2003), and Mathur et al. (2008) observed an age-related decrease in IL-5 stimulated degranulation (Mathur et al., 2008).

2.2. Immunosenescence: dysfunction or adaptation?

The specific age-related changes to cell types of the two arms of the immune system collectively engender immunosenescence and inflammaging. It is well established that elevated inflammation is a risk factor for morbidity and mortality (Brüünsgaard and Pedersen, 2003). Nevertheless, a recent review by Fulop et al. (2017) argues that some immune system changes are beneficial and that the interplay between immunosenescence and inflammaging is more complex than conventionally described.

Fulop et al. (2017) propose that healthy aging entails an optimization of changes encompassing adaptive immune system remodeling, inflammaging, and anti-inflammaging. Changes to the adaptive immune system may serve to optimize resource allocation for changing needs of the organism. For example, thymic involution may decrease the TCR repertoire and the naive T cell compartment, but Fulop et al. (2017) note that it may benefit the organism by lowering energy consumption and that age-related increases of the memory T cell compartment may help the organism combat cognate pathogens and stave off infection. Furthermore, the increase in the Treg compartment may address a growing need for autoimmunity. Although inflammaging may contribute to disease, Fulop et al. (2017) suggest that when optimized, inflammaging may counterbalance the altered ability to fight off new infections brought about by adaptive remodeling, and that anti-inflammaging can serve to prevent inflammation from becoming excessive and destructive.

Other researchers have also argued for careful consideration of age-related changes to the immune system before they are deemed harmful, as many may be beneficial. For example, an increasing proportion of $CD8^+$ T cells are absent of CD28 with age, and Arosa (2002) notes that increased proportions of $CD8^+28^-$ T cells accompany more than 20 conditions and disorders. Although $CD8^+28^-$ T cells have been conventionally considered dysfunctional, terminally differentiated cells, a recent review argues that these cells are involved in tissue repair and homeostasis (Arosa et al., 2016). Thus, the age-related increase in $CD8^+28^-$ T cells, rather than being harmful, may serve to address the increasing need for tissue maintenance of the senescing organism.

Lineage skewing of immune cell compartments is a major characteristic of immunosenescence, which helps give rise to the remodeling of the immune system described above. These changes are involved in complex outcomes including healthy vs. unhealthy aging as well as numerous disease states. These complex outcomes vary markedly between elderly individuals. DNAm and DNAhm can help build on our understanding of immunosenescence because numerous studies have observed associations of DNAm and DNAhm with lineage skewing, inflammation, age and many of the complex outcomes related to aging. We discuss these studies in the sections below and argue that future work on immunosenescence stands to benefit by investigating potential roles of DNAm and DNAhm.

3. Age-related DNA methylation and DNA hydroxymethylation

DNAm has been shown to robustly associate with human age, and this association has been observed across various tissues, with a large number of studies reporting epigenome-wide associations in human whole blood (Alisch et al., 2012; Bell et al., 2012; Bjornsson et al., 2008; Christensen et al., 2009; Teschendorff et al., 2010). During childhood, thousands of CpG sites undergo rapid DNAm changes with age, some of which become hypomethylated and others hypermethylated. The majority of these sites show significant, but less rapid DNAm changes in adulthood (Alisch et al., 2012). Gentilini et al. (2012) observed 13 hypermethylated and 15 hypomethylated sites in offspring of centenarians compared to offspring of non-centenarians suggesting DNAm not only associates with age, but senescence. In addition to nuclear DNAm, mitochondrial DNAm also associates with age and may be a biomarker of aging in both humans and murine models (D'Aquila et al., 2015; Iacobazzi et al., 2013).

To take advantage of the robust association with DNAm and age, several DNAm-based biomarkers of aging have been recently developed. Some of these epigenetic clocks can estimate age across numerous human tissues with high accuracy (Horvath, 2013) and predict outcomes such as mortality more accurately than chronological age alone (Hannum et al., 2013; Horvath, 2013; Levine et al., 2018; Marioni et al., 2015), suggesting it could detect healthy vs. unhealthy aging. Using Horvath's Clock, semi-supercentenarians (N = 82) were observed to have an epigenetic age of 8.6 years younger than their chronological age, and offspring of semi-supercentenarians (N = 63) to be 5.1 years younger than age-matched controls (N = 47) (Horvath et al., 2015). This study demonstrates that DNAm can be used to study complex outcomes of immunosenescence, namely, healthy vs. unhealthy aging, and a recent review highlights the possibility of DNAm as a therapeutic

target to increase longevity (Xiao et al., 2016). The accuracy of DNAm-based predictors as well as the stability of DNAm also allows for the estimation of age in anthropological and forensic contexts. Pedersen et al. (2014) were able to estimate age of a 4000-yr-old Paleo-Eskimo using DNAm from hair tissue, and Giuliani et al., 2016 were able to predict age with high accuracy using DNAm levels extracted from the cementum and pulp of modern teeth based on CpG sites from the genes *ELOVL2, FHL2, and PENK*.

Many loci are consistently correlated with age across multiple studies. The best-known of these loci is DNAm in the CpG island of *ELOVL2*, which has been observed among the top significant hits from numerous epigenome-wide association studies of age (Florath et al., 2014; Gopalan et al., 2017; Karpf, 2012; Marttila et al., 2015; Reynolds et al., 2014; Rönn et al., 2015; Slieker et al., 2018; Steegenga et al., 2014). In a large cohort of 501 subjects aged 9 to 99 years, a Spearman's correlation of 0.92 between age and DNAm was observed in the CpG island of *ELOVL2* (Garagnani et al., 2012). In vitro cell replication experiments demonstrated that *ELOVL2* associated with cell replication rather than senescence and no association between longevity/mortality and whole blood DNAm was observed from participants of the Leiden Longitudinal Study (N = 994; 89–104 years) (Bacalini et al., 2017). These observations suggest that DNAm of *ELOVL2* marks age, but not necessarily aging.

Among the epigenetic biomarkers of age discussed in this section, some are more sensitive to cell lineage skewing than others. Horvath's clock (Horvath, 2013) was designed to be tissue-independent and is thus largely unaffected by cell lineage skewing in blood samples and can predict DNAm across various cell types with similar accuracy. An earlier aging clock (Hannum et al., 2013) was constructed based on blood DNAm and may partially reflect cell lineage skewing, but its inclusion of extrinsic information beyond DNAm may result in more accurate predictions of mortality and health outcomes (Horvath and Raj, 2018). Based on this idea, a third biomarker, DNAm PhenoAge, was constructed to intentionally model extrinsic information such as cell count, inflammatory markers, and other clinical measures, that may inform health outcomes (Levine et al., 2018). The increased success of these biomarkers at predicting mortality is possibly indicative of the importance to aging and mortality of immunosenescent-related processes, such as cell lineage skewing, as well as other extrinsic factors, such as disease state.

In contrast to DNAm, preliminary studies are just beginning to characterize associations between DNAhm and age. DNAhm has been observed to associate with age in the hippocampus and cerebellum of mice aged 7 days, 6 weeks, and 1 year (Szulwach et al., 2011). In human mesenchymal stem cells of 11 young (aged 2–29 years) and 6 old (63–89 years) donors, nominally significant hyper-DNAhm was reported at 785 CpG sites and hypo-DNAhm at 846 CpG sites (Toraño et al., 2016). However, this study did not observe genome-wide significant associations, so larger studies will be needed to assess the extent to which DNAhm associates with age in humans.

4. DNA methylation and hydroxymethylation in lineage skewing

Variation in levels of DNAm across cell types of whole blood at thousands of CpG sites embody cell type specific signatures sufficiently distinct that they are widely used to estimate proportions of blood cell types in human whole blood samples (Houseman et al., 2012; Reinius et al., 2012). A recent study of multiple cancers and inflammatory diseases used DNAm as a marker of lineage skewing, utilizing its ability to measure proportions of myeloid and lymphoid cells in human blood samples, and ultimately demonstrating a pattern of skewing toward the myeloid lineage in a number of diseases (Li et al., 2014). In an analysis of whole genome bisulfite sequencing from 112 samples from BLUEPRINT, DNAm was observed to distinguish between myeloid and lymphoid lineages and patterns of DNAm became more pronounced throughout B and T lymphocyte development (Schuyler et al., 2016). DNAm is therefore useful to measure immunosenescence-related lineage skewing.

Since cell lineage skewing is a well known feature of immunosenescence, one could posit that the observation of age-related DNAm in whole blood is solely attributable to this skewing. This argument, however, would imply that associations between DNAm and age will not be observed within individual blood cell types, which is not the case. In the Multi-Ethnic Study of Atherosclerosis (MESA), age-associated DNAm was observed for 2,595 CpG sites in $CD4^+$ T cells (N = 227) and 37,911 sites in monocytes (N = 1,264), a subset of which associated with age-related expression of genes involved in antigen processing and presentation (Reynolds et al., 2014). A study in human $CD8^+$ T cells isolated from 50 young (22–34 years) and 50 old (73–84 years) individuals observed differential DNAm related to both age and skewing, further demonstrating that one does not confound the other (Tserel et al., 2015). Further, fewer age-differentially methylated CpG sites were observed in peripheral blood leukocytes (806 sites) compared to $CD4^+$ T cells (12,275 sites) and $CD8^+$ T cells (48,876 sites) from the same individuals.

Age-related DNAm changes also accompany shifts in T cell subsets, which are an important feature of immunosenescence. In Tserel et al. (2015), levels of DNAm at CpG sites within several genes involved in regulation of T cell lineage (*CD27, CD248, SATB1, TCF7, BCL11B, RUNX3*) inversely correlated with expression of these genes, and genes involved in immune response were observed to have decreased DNAm and increased gene expression with age. A CpG site in the promoter region of *IFN*-γ has been observed to become hypomethylated during T_h2 polarization in mice (Jones and Chen, 2006). Furthermore, *IL-4* undergoes demethylation within differentiating mouse T_h2 cells (Lee et al., 2002). An age-related decrease in the ratio of T_h2 to T_h1 cells has been observed with age in humans (Uciechowski et al., 2008), for which the results of Lee et al. (2002) and Jones and Chen (2006) provide possible mechanisms involving DNAm.

DNAm changes are potentially involved in Treg dysfunction and may contribute to immunosenescence (Jasiulionis, 2018). Johnson et al. (2017) observed age-related DNAm with an accelerated rate of increase in DNAm in late life at 2 CpG sites in the *KLF14* promoter in human whole blood and replicated this finding in several other human tissues including monocytes and T cell subsets. *KLF14* has been suggested to affect proportions of naive and regulatory T cells via the regulation of *FOXP3* in mice (Sarmento et al., 2015). Rosenkranz et al. (2007) measured the frequency of regulatory T cells in young (23–40 years) and old (51–87 years) individuals and observed significantly higher frequency of regulatory T cells marked by FOXP3 in the old age group (2007). These findings support the interpretation that DNAm changes could influence relative proportions of Treg cell subsets in the elderly.

Although the number of HSCs undergoes a two- to ten-fold increase throughout life in both humans and mice, age-related functional defects in HSCs, including a reduction in self-renewal capacity and the skewing of lineage differentiation, are major factors in immunosenescence (Geiger et al., 2013). Several studies support the interpretation that DNAm and DNAhm are involved in cell lineage skewing of HSCs. In mice, Beerman et al. (2013) observed an age-related reduction in functional potential of transplanted HSCs, measured by the ability of the transplanted cells to reconstitute irradiated bone marrow, and transplants of aged HSCs exhibited skewing of reconstitution toward the myeloid lineage. In addition, age-related DNAm occurred in genes more highly expressed or exclusively expressed in downstream cell lineages at a much higher frequency compared to genes more highly expressed or exclusively expressed in HSCs. This suggests regulatory consequences of DNAm changes in HSCs that do not occur until HSC differentiation. Comparing young vs. old mice, regions gaining DNAm with age were enriched for regions of open chromatin in lymphoid cells, while regions losing DNAm were enriched for regions of open chromatin in myeloid cells, which is consistent with a DNAm-driven

skewing toward the myeloid lineage with age (Beerman et al., 2013). In transplantation experiments of DNMT3A-null and control HSCs in the bone marrow of mice, Challen et al. (2011) found that DNMT3A-null HSCs had higher self-proliferation capacity and lower differentiation capacity with a skewed contribution to peripheral blood of B cells compared to controls. Both hypo- and hyper-methylation were observed at sites in DNMT3A-null HSCs with hypermethylation accounting for 95% of the differential methylation occurring in CpG islands. Many genes involved in HSC differentiation were also downregulated in DNMT3A-null HSCs. Evidence also suggests a role for DNAhm in skewed differentiation of HSCs. In bone marrow genomic DNA of 88 patients with myeloid malignancies with *TET2* mutation and 17 healthy controls, 5hmC levels negatively associated with *TET2* mutation (Ko et al., 2010). This same study observed that in HSCs isolated from mice, downregulation of *TET2* via transduction with *TET2* short hairpin RNA skewed lineage differentiation toward the monocyte/macrophage lineage (Ko et al., 2010). Examination of a mouse model of *TET2* loss yielded similar results, showing that *TET2* loss-of-function mutation resulted in decreased 5hmC content, increased HSC self-renewal capacity, and skewing toward the myeloid lineage (Moran-Crusio et al., 2011). Overall, these findings suggest that DNAm and DNAhm could play a role in immunosenescence, specifically, HSC aging.

5. DNAm in inflammation

A meta-analysis of 8863 individuals of European ancestry found strong evidence that DNAm associates with levels of an inflammatory factor, observing that DNAm at 218 CpG sites associates with CRP levels and replicating 58 of these CpG sites in an African-American panel (n = 4111) (Ligthart et al., 2016). The most significant CpG site (cg10636246) is within 1500 bp of a gene called Absent In Melanoma 2 (*AIM2*), which is expressed in adult peripheral blood $CD27^+$ B cells at steady state (Svensson et al., 2017). In macrophages, AIM2 is involved in an inflammasome response. In particular, in response to human cytomegalovirus (CMV) infection, macrophages made to be deficient of *AIM2* by use of small interference RNA (siRNA) had an impaired ability to induce an inflammasome response (indicated by IL-1β and IL-18), initiate cell death (indicated by Lactate Dehydrogenase), and curb the CMV life cycle (Huang et al., 2017).

Associations between DNAm and inflammatory factors have also been observed in studies of traits related to inflammation such as obesity. Studies investigating obesity and related conditions have reported dysregulation of DNAm associated with inflammatory factors. Yusuf et al. (2017) measured changes in plasma concentrations of patients with dyslipidemia pre- vs. post-intervention with a PPAR-α inhibitor, and observed concentrations of CRP, IL-2, and IL-6 to associate with DNAm at several CpG sites in $CD4^+$ T cells (2017). In PBMC samples of 186 overweight/obese subjects, PM10 (particulate matter 10 μm and smaller) exposure negatively associated with DNAm of *CD14* and *TLR4* (Cantone et al., 2017). In a similar study of 165 obese subjects, DNAm of *TNF-α* negatively associated with nutrient intake of cholesterol, folic acid, β-carotene, carotenoids, and retinol in whole blood (Bollati et al., 2014).

Jasiulionis (2018) review a number of studies suggesting that DNAm mediates the effect of many environmental and lifestyle factors on aging. Some of these studies indicate that an enhanced inflammatory response results from promoter demethylation of *IL-6* in response to deficiency of zinc (Wong et al., 2015). A review by Haase and Rink (2009) suggest that the age-related decrease of zinc may play a role in inflammaging (Haase and Rink, 2009). DNAm may also be involved in inflammation associated with sociocultural and psychological factors. It is well known that living in disadvantaged neighborhoods is associated with poor health outcomes (Diez Roux and Mair, 2010). In monocytes purified from blood from 1,226 MESA participants, socioeconomic disadvantage and neighborhood social environment both associated with DNAm near a number of genes coding for inflammatory factors and in some cases associated changes in gene expression of these factors were also observed (Smith et al., 2017). In the same cohort, both low adult socioeconomic status and low social mobility associated with DNAm near several inflammation-related genes, which in turn associated with gene expression (Needham et al., 2015). In blood samples from participants of the EPIC Italy prospective cohort study (n = 857), socioeconomic status associated with differential DNAm of probe sites within the gene body, the 5' untranslated region, or within 1500 bp upstream of the transcription start site of six inflammation-related genes (Stringhini et al., 2015). In addition to sociocultural factors, a study examining the involvement of epigenetics in psychological phenotypes found that anxiety, depression, and hostility were observed to associate with human whole blood DNAm in the promoter of inflammation-related factors Intercellular Adhesion Molecule-1 (*ICAM-1*) and coagulation factor III (*F3*) (Kim et al., 2016). In whole blood samples from participants of the Grady Trauma Project, post-traumatic stress disorder (PTSD) associated with DNAm of CpG sites within genes involved in inflammation. In the same study, PTSD also associated with plasma concentrations of several factors involved in immune system regulation including several interleukins and TNF-α (Smith et al., 2011).

DNAm changes also accompany changes to inflammatory factors that potentially relate to cardiovascular health in the elderly. In whole blood samples taken from 789 Normative Aging Study (NAS) participants aged 55–100 years, diastolic blood pressure negatively associated with DNAm of *IFN-γ* and positively associated with DNAm of toll-like receptor 2 (*TLR2*) and inducible nitric oxide synthase (*iNOR*) (Alexeeff et al., 2013). Age-related reductions in expression of *TLRs 1-9* as well as nitric oxide synthase has been observed in murine macrophages (Kissin et al., 1997; Renshaw et al., 2002). Another study of NAS participants found that decreased DNAm in LINE-1 repetitive elements associated with increased levels of VCAM-1 in serum samples (Baccarelli et al., 2010). VCAM-1 is an adhesion molecule on endothelial cells that binds lymphocytes (Osborn et al., 1989).

In breast cancer patients, chemotherapy was associated with decreased levels of DNAm at 8 CpG sites, all of which significantly or suggestively associated with levels of inflammatory factors sTNFR2 and IL-6 (Smith et al., 2014). The largest difference in DNAm was observed in 4 CpG sites in exon 11 of transmembrane protein 49, *TMEM49*. These four sites (though not the other four) also associated with CRP levels in the meta-analysis discussed above (Ligthart et al., 2016), suggesting hypomethylation of these sites as a marker of general inflammation.

Changes to DNAm and DNAhm accompany many of the types of changes that characterize immunosenescence. Further study is needed to ascertain the extent to which these epigenetic changes may mediate immunosenescence. We can gain additional insight about possible roles of DNAm and DNAhm in immunosenescence by drawing upon the literature on disease epigenetics, considering that many diseases are marked by inflammation and share overlapping characteristics with immunosenescence.

6. DNA methylation and hydroxymethylation in chronic diseases

Chronic diseases constitute a complex array of outcomes resulting from aging and immune system dysfunction. In this section, we discuss associations of DNAm and DNAhm with various chronic diseases and how they may relate to immunosenescence.

6.1. DNAm and DNAhm in cognitive decline

Changes in 5mC and 5hmC have been reported in studies of age-related cognitive decline. Irier et al. (2014) observed environmental enrichment via the addition of plastic tubes and toys to reduce global 5hmC in the hippocampus and improve cognitive function in aged mice. In the Senescence Accelerated Mouse P8 (SAMP8), environmental

enrichment also improved memory and cognition, which was accompanied by global decreases in 5hmC and increases in 5mC levels, along with decreases in expression of *IL-6* and *CXCL10* (Griñan-Ferré et al., 2016). CXCL10 is involved in the chemotaxis of mononuclear cells (Fife et al., 2001). Chemotaxis refers to the migration of cells toward increasing or decreasing concentration of a chemical, which, in the case of the immune system, is often released at the site of an infection or injury. These results are consistent with the interpretation that 5mC and 5hmC and attenuated inflammaging are involved in the mitigation of neurodegenerative decline (Griñan-Ferré et al., 2016). Furthermore, mice whose cerebra are chronically hyperfused are used to model age-related cerebrovascular degeneration in humans. Compared to controls, the corpus callosum of hyperfused mice had increased levels of both 5hmC and Iba1-positive inflammatory microglia, but association between 5hmC and Iba1-positive inflammatory microglia was observed in both treatment groups (Tsenkina et al., 2014). Iba1 is specifically secreted by and involved in the activation of microglia, which are macrophages residing in the central nervous system (Greter and Merad, 2012; Ito et al., 1998). Involvement of DNAm and DNAhm in Alzheimer's disease (AD) has also been reported. In brain tissue of 460 individuals diagnosed with AD and 263 controls, differential DNAm between cases and controls was observed in 11 DMRs and associations were observed between DNAm and expression of 8 proximal genes, including the gene coding for RHBDF2 (De Jager et al., 2014), a protein observed to be necessary for the transport of TNF-α in mouse macrophages (Adrain et al., 2012). TNF-α is a molecule of considerable scientific and clinical interest that, to date, has been found to be involved in multiple signaling pathways, inflammation, immunity, and human diseases (Chen and Goeddel, 2002; Wajant et al., 2003). In mice used to model AD pathogenesis, a global decrease of 5hmC in hippocampus was observed (Shu et al., 2016). Differential DNAhm between post-mortem brain samples of individuals with Alzheimer's diseases (n = 5) and controls (n = 5) was observed in 325 genes (Bernstein et al., 2016).

6.2. DNAm and DNAhm in cancer

It has been long established that altered DNAm is observed in numerous cancers. Weisenberger (2014) notes several studies reporting that regions of repetitive elements, regions with low density of CpG sites, and lamin-associated domains are hypomethylated, while specific loci in CpG islands and shores are hypermethylated in human cancers, and tumor suppressor genes are often silenced due to hypermethylation of their promoter regions (Baylin, 2005). As of January 2, 2014, The Cancer Genome Atlas enumerated 30 cancers characterized by DNAm alterations in humans (Weisenberger, 2014). To date, DNAhm alterations have also been observed in at least 12 human cancer types, most of which are characterized by decreased 5hmC compared to controls as well as a decrease in 5hmC as the cancer progresses, and several studies have proposed that DNAhm could be used as a tool for cancer diagnosis and prognosis (Bhattacharyya et al., 2013; Chapman et al., 2015; Dong et al., 2015; Jäwert et al., 2013; Ko et al., 2010; Kroeze et al., 2014; Larson et al., 2014; Lian et al., 2012; Liao et al., 2016; Liu et al., 2013; Müller et al., 2012; Song et al., 2017; Thomson and Meehan, 2017; Yang et al., 2013; Ye and Li, 2014; Zhang et al., 2015).

DNAm and DNAhm may also be involved with inflammation in cancers. Using cell type specific DNAm microarray data to identify signatures of differential DNAm between myeloid and lymphoid cells, a study of multiple cancers noted that differential DNAm patterns observed in whole blood samples of cancer patients vs. controls were consistent with shifting cell populations: specifically, an increase of myeloid cells and a decrease of lymphoid cells within cancer patients. The study also noted high levels of overlap between whole blood DNAm changes observed in cancer and those observed in inflammatory diseases (Li et al., 2014). In blood samples of participants from The Normative Aging Study (NAS; n = 795), promoter hypermethylation of *IFN-γ*, *ICAM-1* and *IL-6* was observed to associate with prostate cancer incidence, and promoter hypermethylation of *IFN-γ* also associated with all-cancer incidence (Joyce et al., 2015). DNAhm is also suggested to play a role in the infiltration of immune cells into melanoma (Fu et al., 2017).

6.3. DNAm and DNAhm in non-cancerous diseases

Differential DNAm and DNAhm have been observed in non-cancerous chronic diseases as well. Levels of DNAm and DNAhm have been quantified between healthy individuals and those with chronic diseases across various tissues including peripheral blood mononuclear cells (PBMCs), brain, liver, heart, colon, and spinal cord, among others. Investigators have collected cell subtypes from these tissues, such as $CD4^+$ T cells from PBMCs and frontal cortex tissue from the brain. A representative selection of these studies is presented in Supplementary Table 1. Notably, many of the diseases showing robust associations with DNAm involve an inflammatory component and may share processes with immunosenescence, suggesting that the wealth of information generated by these studies could inform current knowledge on the relationship between DNAm, DNAhm, and immunosenescence.

Some inflammatory diseases are characterized by hundreds of disease-associated DNAm sites in blood: an example of this is inflammatory bowel disease (IBD). Karatzas et al. (2014) reviewed studies relating DNAm to IBD and its two principal subtypes Crohn's disease (CD) and ulcerative colitis (UC) in blood and other tissues, and noted IBD-associated DNAm in 19 genes, CD-associated DNAm in 79 genes, and UC-associated DNAm in 91 genes. A systematic review focused on genes linked to inflammatory response identified 25 genes differentially methylated between UC cases and controls or UC inflamed and quiescent mucosa (Gould et al., 2016). More recently, a study comparing DNAm in PBMCs of 240 patients newly-diagnosed with IBD and 190 controls, found 439 sites to be differentially methylated, with nearby genes showing enrichment for immune function. This study also identified three differentially methylated regions which replicated in an independent cohort and covered the genes *TXK, ITGB2*, and *VMP1*. The authors observed IBD-associated hypermethylation of *TXK* promoter DNAm, which associated with reduced expression of *TXK* in whole blood and $CD8^+$ T cells (Ventham et al., 2016). *TXK* expression is involved in the development of human T helper cells (Kashiwakura et al., 1999). *ITGB2*, also known as *CD18*, is involved in leukocyte adhesion (Tan, 2012). As we discuss in section 5, CpG sites in *VMP1*, also known as *TMEM49*, have been reported to associate with chemotherapy and levels of inflammatory factors in humans (Smith et al., 2014), and with human CRP levels in a large meta-analysis (Ligthart et al., 2016). The association of *VMP1* DNAm with multiple conditions and inflammatory factors suggest its involvement in inflammation in general. Notably, Somineni et al. (2019) found 1189 CpG sites (including sites in *VMP1*) to be differentially methylated in children diagnosed with Crohn's disease, but observed that these CpG sites showed an extremely similar signature of association with CRP levels ($r = 0.91$). Moreover, DNAm at these sites reverted to normal levels after treatment, suggesting that the observed differences may reflect a DNAm signature of inflammation rather than Crohn's disease.

5mC and 5hmC has also been reported to be involved in the inflammation and immune system decline in systemic lupus erythematosus (SLE). SLE is an autoimmune disease that typically affects individuals in mid to late life, and shares many characteristics with immunosenescence, most notably in T cells (van den Hoogen et al., 2015). Wu et al. (2016) reviewed studies indicating that there is a global decrease of DNAm in PBMCs, B cells, and $CD4^+$ T cells in SLE, including demethylation of a number of genes encoding CD molecules, cytokines, and pro-inflammatory markers. A recent study used RNA interference to experimentally alter global methylation levels by knocking down *BDH2* expression in $CD4^+$ T cells of SLE patients, and found that the induced hyper-DNAhm and hypo-DNAm resulted in increased expression of autoimmune-related genes such as *CD70*, *CD11a*,

CD40L, and *PRF1* (Zhao et al., 2017b). Overall, this suggests that, in the $CD4^+$ T cells of SLE patients, increased global DNAhm and decreased global DNAm can alter expression of the aforementioned autoimmune-related genes.

Studies investigating other chronic diseases involving an inflammatory response have also found evidence of a relationship between DNAm and inflammation. In white blood cells of patients with chronic obstructive pulmonary disease differential DNAm was observed at 349 CpG sites near genes enriched for immune and inflammatory system pathways (Qiu et al., 2012). In PBMCs of chronic hepatitis B patients, hyper-DNAm of *PPAR*-γ associated with inhibition of its transcription, and with liver inflammation and fibrosis, although the particular function of *PPAR*-γ in the liver is unclear (Zhao et al., 2013). In fibroblast-like synoviocytes (cells residing in joint cavities) of patients with rheumatoid arthritis compared to those with osteoarthritis, Karouzakis et al. (2014) observed promoter hypomethylation accompanied by high expression of T-box transcription factor (*TBX5*). In a human synovial sarcoma cell line, knocking down the expression of *TBX5* decreased and overexpression of *TBX5* increased production of pro-inflammatory cytokines (Hussain et al., 2018). Collectively, these studies suggest a potential interaction between DNAm and expression of inflammatory genes across a number of inflammatory chronic diseases, although the exact regulatory roles are not yet established. In fibroblast-like synoviocytes isolated from 14 patients with rheumatoid arthritis and 12 patients with osteoarthritis that were stimulated with either IL-1β or TNF, *DNMT3A* expression decreased after stimulation of either cytokine and *DNMT1* expression decreased after IL-1β stimulation (Nakano et al., 2013). While other experimental work in humans has shown evidence of DNAm changes influencing levels of immune-related factors via altered expression (Zhao et al., 2017b), this study raises the possibility that changes in immune-related factors may influence DNAm.

Changes in 5mC and 5hmC have also been observed in inflammation of other tissues, including skin, kidney, and spinal cord. Low global levels of 5hmC were observed in samples from lesional and perilesional skin cells of individuals with an inflammatory skin disease known as hidradenitis suppurativa (n = 30) compared to healthy controls (n = 30) suggesting DNAhm may play a role in skin cell inflammation as well (Hessam et al., 2017). In a study investigating the effect of diabetes on kidney function, decreased mitochondrial 5mC and 5hmC were observed in kidney tissue of streptozotocin-induced diabetic rats and accompanied diminished uric acid clearance (de Oliveira et al., 2017), the lack of which likely contributes to kidney inflammation (Wang et al., 2012). In the spinal cord of mice with formalin-induced acute inflammatory pain, an increase of TET1 and TET3 as well as 5hmC was observed. Moreover, injection of *TET1*-siRNA or *TET3*-siRNA in mice decreased 5hmC and alleviated formalin-induced nociceptive response compared to controls, suggesting that 5hmC may regulate nociceptive behavior (Pan et al., 2016).

Taken together, these findings highlight a potential role of DNAm and DNAhm in immune system decline with age in chronic disease patients, and demonstrate the utility of examining DNAm and DNAhm to better understand immunosenescence in numerous chronic diseases. The majority of studies contributing to our current understanding of immunosenescence have not interrogated DNAm and DNAhm. Other work has observed DNAm and DNAhm to associate with immune-related factors, but has not directly investigated age. Moreover, the majority of results discussed above reflect associations rather than causal relationships. Thus, there are gaps in our understanding of the potential relationship between immunosenescence and these two epigenetic modifications. Below we outline the design of future studies suited to further our understanding of the role of DNAm and DNAhm in immunosenescence.

7. Future directions

Given the DNAm, DNAhm, and gene expression changes reported to occur with aging, disease state, and features of immunosenescence such as lineage skewing, it is tempting to postulate potential causal mechanisms driving immunosenescence. For example, changes to DNAm and DNAhm in HSCs could drive changes to gene expression resulting in altered differentiation and other phenotypic changes to HSCs observed with age. It is possible that many of the immunosenescence-related changes observed in PBMCs are products of changes that begin in HSCs. Alternatively, changes observed in immune cells could originate subsequent to differentiation from HSCs. The mechanisms behind immunosenescence are difficult to disentangle given that the vast majority of studies reviewed here investigate associations, and the experimental studies have shown mixed evidence. The limited evidence establishing causal relationships constitutes a large gap for future studies to fill. Below, we suggest several promising strategies to investigate potential mechanisms underlying immunosenescence.

First, more controlled experimental studies are needed to directly assess potential causal relationships. For example, transplanting young, healthy bone marrow into immunosenescent murine models and observing changes such as a reversal of lineage skewing and a reversion to younger phenotypes within white blood cell lineages, as well as concurrent changes to DNAm, DNAhm, and gene expression within these cell types, could determine whether immunosenescent phenotypes are rooted in age-related phenotypic changes to HSCs. Observing DNAm, DNAhm, and gene expression changes in patients undergoing bone marrow transplants could also be similarly fruitful. Knockouts or siRNAs that target DNMT and TET genes known to manipulate DNAm and DNAhm (i.e. *DNMT1*, *DNMT3a*, *DNMT3b, TET1*, *TET2*, *TET3*), could help establish whether DNAm and DNAhm mediate phenotypic changes characteristic of immunosenescence, or are merely markers of such changes. While these experiments would alter global levels of DNAm and DNAhm, advancements such as CRISPR-Cas9 raise the possibility of experimentally manipulating DNAm and DNAhm at specific CpG sites (Hsu et al., 2014; Liu et al., 2016).

As a complement to experimental approaches, analytical approaches such as Mendelian Randomization (MR) have been proposed that are capable of making causal inferences from existing epidemiological data (Davey Smith and Hemani, 2014). While such methods are not substitutes for controlled experiments, they can be easily applied to human studies, can generate discoveries to be further validated through experimentation, and can take advantage of the wealth of large genomic datasets generated over the last decade. There are many publicly available datasets to which this approach could be applied. For example, the original Framingham Heart Study is a longitudinal cohort of 5209 participants of a wide age range with stored blood samples and an extremely rich set of adjudicated disease phenotype data (Mahmood et al., 2014). Application of MR to large datasets enables inference on whether locus-specific DNAm that associates with the expression of an immune-related factor is 1) causal or 2) consequential to changes in expression of the factor. Such inferences directly address the question of whether these epigenetic modifications regulate immunosenescence or simply mark it. MR relies on the assumption that alleles are passed to offspring independently of potential confounders such as environmental exposures. This assumption allows the use of genetic variants as instrumental variables that can be used to mimic randomization of levels of DNAm or immune factors. MR can thus be used to establish directional causal relationships between site-specific DNAm and immune-related factors, and to distinguish causal relationships from the situation where both DNAm and immune factors are influenced by a third confounding factor (Burgess et al., 2015).

Single cell sequencing is another approach that does not require an experimental setting that could further elucidate regulatory relationships in immunosenescence. For example, within a tissue sample, it could be the case that some cells have decreased locus-specific DNAm upregulating expression of an immune factor while other cells have increased DNAm at the same locus downregulating expression of the same factor. If DNAm and gene expression signals were measured across a cell population, these signals would cancel each other out.

Table 1
Summary of characteristics of immunosenescence and associated epigenetic changes.

Characteristic	Description	Current understanding of connection with DNAm	Current understanding of connection with DNAhm
Aging (Section 3)	The general deterioration of the organism associated with an increased risk of morbidity and mortality.	Evidence of widespread site-specific changes in human blood cells and other tissues	Preliminary evidence of region- and site-specific changes in mouse and human brain tissue
Lineage skewing (Section 4)	Skewing toward the myeloid lineage in HSCs	Evidence of causal role for DNAm in HSC differentiation. (Challen et al., 2011)	Evidence for causal role of DNAhm in skewing toward myeloid lineage of HSCs (Ko et al., 2010; Moran-Crusio et al., 2011).
	Changes in T cell subsets	Site-specific changes (Lee et al., 2002; Jones and Chen, 2006;Tserel et al., 2015)	Uncharacterized
Inflammaging (Section 5)	Increase in stably low levels of inflammation	Numerous site-specific associations observed with inflammation and inflammatory diseases	Associations found in some inflammatory diseases Evidence of causal role in inflammatory pain (Pan et al., 2016)
Chronic diseases (Section 6)	Chronic diseases can be viewed as complex outcomes of aging that vary markedly by severity and tissue affected.	Evidence of causal role in SLE (Zhao et al., 2017b) Associations with numerous chronic diseases	

With single cell sequencing of both the methylome and transcriptome, however, such signals would be detectable (Clark et al., 2018; Smallwood et al., 2014). Similar arguments can be applied to DNAhm of immune-related factors.

Each of these approaches has the potential to reinforce each other. The advantage of controlled experiments is that tissues and cell types can be experimentally manipulated to more directly interrogate immunosenescence-related processes, holding constant potential confounding factors. The advantage of the other approaches is that they may be applied to human studies, since they can use existing data from human tissue samples. MR has the potential to draw causal inferences at specific sites, while RNA interference approaches to manipulate levels of DNA methyltransferases and TET proteins allow inference of causal effects of changes in global levels of DNAm and DNAhm. However, if MR studies demonstrate evidence of epigenetic regulation of expression of particular genes involved in immunosenescence, RNA interference could be used to investigate the impact of knocking out those genes on the expression of other genes, identifying potential downstream pathways. Further, as the technology becomes more widely adapted, CRISPR-Cas9 can be used to validate causal inferences by experimentally manipulating DNAm and DNAhm at specific CpG sites. Therefore, a coordinated effort involving all of these strategies has the potential to be particularly fruitful.

8. Conclusion

Numerous studies support the interpretation that DNAm and DNAhm play a role in immune system decline, most notably in white blood cell types and HSCs. Here, we have highlighted findings of DNAm/DNAhm changes associated with many features of immunosenescence, including lineage skewing, inflammatory factors, aging, and disease state (Table 1). However, for the most part the causal relationships underlying these associations and defining the role of DNAm/DNAhm in immunosenescence remain to be elucidated. To fill this gap, we have proposed a multi-pronged approach involving both experimental and observational studies to further our understanding of the specific roles DNAm and DNAhm may play in immunosenescence.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.arr.2019.01.011.

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