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DNA Damage, Metabolism and Aging in Pro-Inflammatory T Cells Rheumatoid Arthritis as a Model System

Yinyin Li, Jörg J. Goronzy, and Cornelia M. Weyand*

Department of Medicine, Division of Immunology and Rheumatology, Stanford University, Stanford, CA 94305 and the Department of Medicine, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94306

Abstract

The aging process is the major driver of morbidity and mortality, steeply increasing the risk to succumb to cancer, cardiovascular disease, infection and neurodegeneration. Inflammation is a common denominator in age-related pathologies, identifying the immune system as a gatekeeper in aging overall. Among immune cells, T cells are long-lived and exposed to intense replication pressure, making them sensitive to aging-related abnormalities. In successful T cell aging, numbers of naïve cells, repertoire diversity and activation threshold are preserved as long as possible; in maladaptive T cell aging, protective T cell functions decline and proinflammatory effector cells are enriched. Here, we review in the model system of rheumatoid arthritis (RA) how maladaptive T cell aging renders the host susceptible to chronic, tissue-damaging inflammation. In T cells from RA patients, known to be about 20 years pre-aged, three interconnected functional domains are altered: DNA damage repair, metabolic activity generating energy and biosynthetic precursor molecules, and shaping of plasma membranes to promote T cell motility. In each of these domains, key molecules and pathways have now been identified, including the glycolytic enzymes PFKFB3 and G6PD; the DNA repair molecules ATM, DNA-PKcs and MRE11A; and the podosome marker protein TKS5. Some of these molecules may help in defining targetable pathways to slow the T cell aging process.

Keywords

T cell aging; DNA damage responses; inflammation; rheumatoid arthritis; mtDNA; ATM; DNA-PKcs; MRE11A; telomere

*Corresponding Author at: Department of Medicine, Division of Immunology and Rheumatology, Stanford University School of Medicine, 269 Campus Drive West, Stanford, CA 94305-5166, USA. cweyand@stanford.edu (C. M. Weyand).

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Introduction

The DNA in each of the trillions of cells in our body is under constant assault by exogenous and endogenous toxins, genotoxic chemicals and cellular metabolism inducing damage to the genetic material (Ciccia and Elledge, 2010; Lindahl and Barnes, 2000). To maintain DNA integrity, cells elicit a highly specific intracellular and intercellular response network to sense, signal and repair DNA lesions, thus preventing the generation of nucleotide alterations, single-strand breaks and double-strand breaks (Jackson and Bartek, 2009). The conglomerate of hierarchical pathways protecting DNA integrity has been termed DNA damage response (DDR). Declining efficiency of DNA repair systems will lead to the accumulation of damaged DNA which causes functional failure or cell death. Notably, DNA damage accumulation or repair system defects promote cellular senescence or apoptosis (Childs et al., 2015). Thus, preserving DNA intactness is a cardinal feature of health and a decline in DDR is considered a major contributor to organismal aging (Pan et al., 2016). Aging is characterized as a time-dependent progressive functional decline, leading to loss of organismal homeostasis, increased human pathologies and eventually death (Fulop et al., 2011; Lopez-Otin et al., 2013). The decline of immune protective adeptness, often referred to as immune aging, is one of the key processes associated with advancing age and contributes to numerous age-related morbidities. Immune aging impairs the host's protection against pathogen invasion, renders the host susceptible to malignancies, weakens wound healing and tissue repair, while increasing the risk for chronic inflammation and autoimmune disease development (Goronzy et al., 2013; Goronzy and Weyand, 2013; Palmer, 2013). Immune system aging is one of the key components leading to inflammaging; a process of smoldering inflammation in the elderly, but other age-related changes, such as accumulation of infections and senescent cells, metabolic syndrome, etc may also contribute.

In patients with rheumatoid arthritis (RA), the immune aging process is accelerated; best captured in prematurely aged T cells that loose CD28, have shortened and damaged telomeres, impaired DNA repair systems and excessive production of cytokines (Goronzy and Weyand, 2012; McInnes and Schett, 2007; Schmidt et al., 1996b; Weyand et al., 2014). RA T cells are metabolically reprogrammed, affecting their differentiation, function, and longevity (Weyand et al., 2017). In this review, we will use the inflammatory syndrome RA as a model system to discuss how DNA damage accelerates T cell aging and how old T cells promote and sustain tissue inflammation. Defining molecular targets at the interface of immune cell aging and inflammation may allow the development of novel interventions counteracting the detrimental effects of organismal aging and inflammatory disease.

Maladaptive T Cell Aging in Rheumatoid Arthritis

T cells from RA patients have an array of features that identifies them as being prematurely aged: the accumulation of CD28⁻ effector T cells, telomere fragility and attrition, inefficient DNA damage repair, metabolic reprogramming, and excess production of cytokines compatible with a senescence-associated secretory phenotype (SASP) (Figure 1). To keep immune homeostasis, e.g. securing population density, diversity and cellular competence, the T cell compartment develops adaptive strategies to generate new T cells, protect long-

term survival of existing T cells and monitor functional adeptness of individual T cells (Goronzy and Weyand, 2017). In mice, survival times of naïve T cells are short and their development entirely dependent on the thymus (Bains et al., 2009; den Braber et al., 2012; Tsukamoto et al., 2009). In contrast, due to the involution of the thymus during the first third of life, humans had to adopt a completely different strategy to generate and maintain their T cell compartment: they generate new T cells by homeostatic proliferation of already selected T cells. Over a lifetime, continuous peripheral proliferation, progressive T cell differentiation and persistent stimulation by infectious agents will eventually build a T cell compartment with contracted diversity, partial cellular differentiation and reduced cellular competence (Czesnikiewicz-Guzik et al., 2008; Moskowitz et al., 2017; Sauce et al., 2012).

The T cell memory compartment, characterized by cell inflation and TCR repertoire shifts, serves the aging host by optimizing anti-viral immunity, particularly to CMV and VZV infections (Derhovanessian et al., 2014; Goronzy et al., 2001; Levin et al., 2003). Changes in the frequencies and composition of regulatory T cell (Treg) are also associated with aging. In older individuals, the population of naïve-like CD4⁺CD45RA⁺CD25⁺ Treg cells is fading and CD4⁺CD45RO⁺CD25^{hi} memory-like Treg cells are increasing (Seddiki et al., 2006; Valmori et al., 2005). Notably, the number of CD4RA⁺CCR7⁺NOX2⁺ naïve-like CD8⁺ Treg cells decreases with age as well (Wen et al., 2016). NOX2⁺ CD8 Treg cells exert anti-inflammatory functions by controlling the size of the CD4 T cell compartment and their age-related decline favors unopposed tissue inflammation. Thus, healthy immune aging results from a multitude of adaptive mechanisms, all geared to optimize immune protection while maintaining homeostasis. In patients with RA, the process of immune aging is maladaptive, resulting in the accumulation of pro-inflammatory effector cells and susceptibility of the host to suffer from unopposed tissue inflammation (Figure 1).

Unfavorable outcomes of immune aging include inefficient protection against infection, impaired vaccine responses, susceptibility to cancer development and compromised wound healing. Studying T cells from RA patients has been informative in exploring the role of old T cells in tissue inflammation. Most of these studies were performed with purified naïve CD4 T cells from RA patients that were stimulated to enter effector cell differentiation. Thus, abnormal phenotypes and functional behaviors should not be a consequence of the inflammatory disease process, as naïve T cells have no role in tissue inflammation. Focusing on the naïve CD4 T cell population, however, has allowed functional and molecular studies prior to such T cells entering the rheumatoid disease process. In this review, reference to RA T cells relates to stimulated naïve CD4 T cells that have converted into effector cells.

An important conceptual progress has been that the aged T cells from RA patients are not in permanent cell cycle arrest and thus do not fulfill the criteria of cellular senescence (Branzei and Foiani, 2008; Fujii et al., 2009; Sharpless and Sherr, 2015). Aged RA T cells remain proliferative and functionally highly active (Fujii et al., 2009). Functional activity of senescent cells has been captured in the concept of the senescence-associated secretory phenotype (SASP). By secreting pro-inflammatory cytokines, such as TNF- α and IFN- γ , old cells can regulate neighboring cells and tissues and eventually contribute to tissue inflammation and autoimmune disease (Weyand et al., 2014). TNF- α and IL-6 are both considered age-related biomarkers (Dobbs et al., 1999) and have been associated with aging-

induced diseases. Also, IL-17 is known for its pro-inflammatory functions and has been implicated in rheumatic disease in both mice and humans (Kryczek et al., 2011; Lubberts, 2010). IL-17 may play a role in bone reabsorption by promoting the differentiation of osteoblasts into osteoclasts through the NF- κ B pathway (Kim et al., 2015). Both, T cells committed to the Th1 and Th17 lineage, producing IFN- γ and IL-17, respectively, have been characterized as key contributors to the pathogenesis of a spectrum of autoimmune diseases.

Recent work has revealed that the functional commitment of RA T cells, driving the persistent inflammation of synovial tissue, is mechanistically connected to two molecular domains: inefficiency of DNA repair and telomere structural instability (Cavanagh et al., 2012; Goronzy et al., 2013; Goronzy and Weyand, 2012) as well as metabolic reprogramming (Weyand and Goronzy, 2017; Weyand et al., 2017; Yang et al., 2015). Metabolic analysis of RA T cells has yielded insights into the connection between fuel, proliferative demand and the utilization of cellular metabolites in supporting the cell's misbehavior. Like healthy T cells, RA T cells use glucose to support their energy needs and the synthesis of biosynthetic precursors. In contrast to T cells from healthy age-matched individuals, T cells from RA patients generate less ATP (due to the repression of the glycolytic enzyme phosphofructokinase; PFKFB3) and produce more NADPH due to the overexpression of glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the pentose phosphate pathway (PPP) (Yang et al., 2013; Yang et al., 2016). The dysbalance between ATP and NADPH generation leads to consumption of reactive oxygen species (ROS) and surplus of reductive elements. Reduction in ROS is directly related to a deviation in T cell differentiation to favor Th1 and Th17 commitment. The overall result is an intracellular milieu biased towards reductive elements and biosynthesis of fatty acids, which are deposited as cytoplasmic lipid droplets. The adaptor molecule TKS5, which connects membrane podosomes with the cytoskeletal machinery, enables T cells to be highly motile and tissue-invasive (Shen et al., 2017). Interestingly, metabolic reprogramming in RA T cells has also been linked to the DNA repair machinery. The DNA repair and cell cycle kinase Ataxia telangiectasia mutated (ATM) is a redox sensor and requires cellular ROS to be activated. In RA T cells, ATM activity is distinctly low, leaving such cells with a defect in DNA repair and allowing the cells to bypass the G2/M cell cycle checkpoint (Shao et al., 2010; Weyand and Goronzy, 2017; Yang et al., 2016). No mutations have been identified in the ATM gene of RA patients. Rather, both transcription of ATM and phosphorylation of the protein are repressed (Yang et al., 2016). Insufficient DNA repair inevitably renders the cell sensitive to aging and to cell death, which will impose proliferative pressure onto the immune system to replenish T cells.

In essence, the interfacing of metabolic status with the DNA repair machinery has major consequences for T cells, accelerating T cell aging through different pathways; e.g. accumulation of unrepaired DNA, cell cycle dysregulation, cell death sensitivity, and lymphopenia-induced proliferative pressure. Aged T cells are not in cell cycle arrest, thus do not fulfill criteria for senescence. Deciphering the maladaptive immune aging process in RA T cells should be helpful in developing a molecular taxonomy of immune aging.

DNA damage response in T cell aging

A growing burden of damaged DNA is considered a hallmark, and a critical inducer, of cellular aging. Multiple DNA damage forms, including somatic mutations, chromosomal aneuploidies and copy number variations accumulate within cells during aging (Kennedy et al., 2012). All of these DNA alterations may affect transcriptional signaling pathways and result in cell dysfunction and immune homeostasis failure. Mutations of DDR genes have been linked to a large variety of diseases and cancer-related syndromes (Jeggo et al., 2016), emphasizing the essential role of DDR networks for health and disease. In older people, DNA damage foci appear in hematopoietic stem cells, indicative of inefficiencies in repairing DNA double-strand breaks (DSBs) (Nijnik et al., 2007; Rube et al., 2011). In human T cells, the frequency of DSBs increases with age and typically more differentiated memory T cells are affected to a higher degree than naïve T cells (Scarpaci et al., 2003). DNA damage may contribute to increased inflammatory responses in the old. In elderly patients with chronic and acute disease, increased DNA damage and cell death give rise to higher cell-free DNA in plasma, and these patients have an increased 9-month mortality (Fournie et al., 1993). Circulating cell-free DNA is considered a potential contributor to tissue damage and inflammation in patients with several autoimmune disorders and correlates with age-associated inflammatory biomarkers (AbdelHalim et al., 2016; Hsieh et al., 2016). However, direct evidence that plasma DNA fragments induce tissue inflammation has not been provided. In eukaryotic cells, multiple cellular processes including cell-cycle progression, DNA replication and DNA repair are tightly coordinated to maintain genomic stability. Cells devote considerable energy and genetic machinery to DDR, which sense and signal problems with DNA integrity, arrest cell-cycle progression and active DNA repair mechanisms. Deficiencies in DNA repair system result in the accumulation of both telomeric DNA and non-telomeric DNA damage in CD4 T cells from patients with RA (Li et al., 2016; Shao et al., 2009), which has been mechanistically linked to T cell aging and chronic tissue inflammation.

ATM

ATM (ataxia-telangiectasia mutated) and ATR (ATM and RAD3-related) are two of the critical regulators in the DNA damage response process. Both of them can be activated by DNA damage and replication stress, but their functions are distinct and not redundant (Marechal and Zou, 2013). ATM is primarily activated by DSBs, whereas a broad spectrum of DNA damage including DSBs and other DNA lesions that interfere with replication can induce ATR activation. Both kinases belong to the phosphatidylinositol-3-OH-kinase-like kinase (PIKKs) family, protein serine-threonine kinases that share sequence similarities with phosphatidylinositol-3 kinases (Lempiainen and Halazonetis, 2009; Lovejoy and Cortez, 2009). ATM activation results in the phosphorylation of a broad spectrum of substrates, including Brca1, Chk2, and p53, which mediates ATM's effects on coordinating multiple cellular responses to DSB repair (Fernandez-Capetillo et al., 2002; Gatei et al., 2000; Kang et al., 2005; Matsuoka et al., 1998; Smolka et al., 2007). In humans, mutations in both alleles of ATM induce a cancer predisposition disorder called ataxia-telangiectasia (A-T), which manifests with abnormalities in the nervous system, the immune system and is

associated with susceptibility to cancer development (Choi et al., 2016; Goldgar et al., 2011; Thompson et al., 2005).

In RA T cells, ATM expression is lower on both the transcript and protein level, but ATR induction appears intact (Shao et al., 2009). This defect results in high DNA damage accumulation, especially in accrual of unrepaired DSB after gamma radiation. RA T cells also are low in the tumor suppressor p53, a downstream effector of the kinase. Reconstitution of p53 in RA T cells improves DNA damage repair capability, but is not able to enhance ATM expression. Conversely, overexpression of ATM is sufficient to restore other members of the DNA repair machinery as well as p53, and eventually rescues all abnormalities in the patients' T cells. RA T cells share many features that are typically found in cells from A-T patients. A-T is considered a progeroid syndrome, with aging related signatures appearing as early as in the second decade of life. T cells from RA and A-T patients share the susceptibility to apoptosis, leading to premature T cell loss, compensatory homeostatic proliferation and eventually to the accumulation of end-differentiated effector T cells (Liuzzo et al., 1999; Schmidt et al., 1996a). In RA patients, such end-differentiated T cells have a CD28^{null} phenotype, form large clonotypes and seek residence in the inflamed joint.

ATM is a highly sensitive sensor for ROS (Zhang et al., 2015); providing a mechanistic link to coordinate the high energy needs of cell proliferation with the necessity to repair DSBs. In RA, naïve CD4 T cells produce low levels of intracellular ROS due to defective glucose utilization (Yang et al., 2016). The underlying abnormality relates to the reduction of the glycolytic enzyme PFKFB3, leading to slow glycolytic breakdown, low production of ATP, low secretion of lactate and insufficient supply of pyruvate to the mitochondria. Unable to enter the glycolytic pathway, glucose in RA T cells is shunted towards the PPP. Here, the underlying abnormality has been mapped to transcript and protein upregulation of G6PD, the rate-limiting enzyme in the PPP. One of the end products of the PPP is NADPH, a critical reductive element in cells. RA T cells accumulate high concentrations of NADPH, followed by enrichment of reduced glutathione. In a ROS-low environment, ATM is insufficiently activated. T cells with insufficiently activated ATM bypass the G2/M cell-cycle checkpoint. ATM^{low} RA T cells rapidly convert from the naïve to the memory state and their enhanced cellular proliferation is associated with preferential differentiation into Th1- and Th17-committed effector cells (Yang et al., 2016).

In addition, ATM has been implicated in innate immunity. In cells from A-T patients and in ATM-deficient mice, unrepaired DNA fragments in the cytoplasm activate type I interferon (IFN) signaling through the stimulator of interferon genes (STING) pathway (Hartlova et al., 2015). STING functions as a cytoplasmic DNA sensor and an adaptor molecule in type I IFN signaling, thus mediating antiviral and antipathogen immune responses. Together with other DNA sensors, STING is also responsive to mitochondrial DNA (mtDNA), thus recognizing mitochondrial stress. In ATM mutant mice, there is strong evidence for an association between mitochondrial failure, oxidative stress and pathologic changes, particularly in Purkinje cells (Borghesani et al., 2000) assigning a regulatory role to ATM in the cytoplasm and not only in the nucleus. Whether ATM^{low} T cells of RA patients have difficulties with cytoplasmic ATM is unknown as they still maintain ATM function. In the

cerebella of completely ATM-deficient mice, upregulation of AMP-activated protein kinase (AMPK) is correlated with ROS elevation and this upregulation is attenuated after treating with the antioxidant monosodium luminol (Luo et al., 2013). In contrast to completely ATM-depleted cells, which appear to degenerate rapidly, ATM^{low} RA T cells still survive over many decades. With only about 50% of ATM activity available, RA T cells need to adapt to new regulatory pathways and need to find ways to survive energy stress and deal with unrepaired DNA.

In essence, ATM's kinase activity plays a critical role in multiple cellular processes; repair of DSBs, control of cell-cycle progression, handling of cytoplasmic DNA fragments, sensing of mitochondrial intactness. Accordingly, ATM coordinates the energy needs imposed by cellular duplication with securing an intact genome and is centrally placed in the cell's aging processes. ATM^{low} RA T cells age prematurely and display pro-inflammatory effector functions (Figure 2).

DNA-PKcs

Like ATM, DNA-PK (DNA-dependent protein kinase) belongs to the phosphatidylinositol 3-kinase-related kinase protein family and contributes critically to the repair of DSBs, including repair activity required in V(D)J recombinations. The serine/threonine protein kinase is composed of the catalytic subunit DNA-PKcs and a heterodimer of Ku proteins containing Ku70 and Ku80 (Smith and Jackson, 1999). Besides its role in the DNA DSBs repair machinery, DNA-PKcs has been implicated in modulating chromatin structure and maintaining telomeres (Gilley et al., 2001). The best studied function of DNA-PKcs is the repair of DNA DSBs through non-homologous end joining (NHEJ) and homologous recombination (HR) (Lieber, 2010). Recent reports have suggested that DNA-PKcs may interfere with the regulation of insulin sensitivity by transcriptional regulation of lipogenesis-related genes (Wong et al., 2009). Also, DNA-PKcs participates in sensing and adjusting cellular energy homeostasis through one of its targets, AMP-activated protein kinase (AMPK). AMPK acts as a sensor of AMP/ATP ratios, thus ultimately guiding glucose consumption and metabolic reprogramming. DNA-PKcs interacts with the AMPK- γ subunit, and its deficiency decreases AMPK phosphorylation induced by glucose-deprivation (Amatya et al., 2012). The involvement of a major DNA repair molecule in regulating cellular energy production again emphasizes the coordination between DNA replication, DNA repair, cell cycle progression and the cell-endogenous production of ATP and biosynthetic precursor molecules.

Just like the two other PIKK family members ATM and ATR, DNA-PKcs has also been linked to immune aging and human diseases. Germline mutations of DNA-PKcs in mice lead to severe combined immunodeficiency (SCID), and selected somatic mutations or small molecule inhibitors that inactivate DNA-PKcs kinase activity induce defects in DSB repair and promote genomic instability (van der Burg et al., 2009). Tissue expression of DNA-PKcs is associated with increased risk of death and inversely correlates with overall survival in cancer patients, possibly related to the kinase's involvement in creating an energy-rich environment encouraging tumor development and growth (Goodwin and Knudsen, 2014). A possible role of DNA-PKcs in innate immunity and inflammation has been suggested.

Specifically, DNA-PKcs seems to be able to signal through the NF- κ B pathway, activating the adhesion molecule VCAM and the inflammatory cytokine TNF (Ju et al., 2010).

Like ATM, DNA-PKcs can bind to DNA fragments from various pathogens to stimulate the STING-dependent pathway, resulting in IRF3 activation and ultimately the release of cytokines and chemokines (Ferguson et al., 2012). Notably, DNA-PKcs depletion abolishes DNA-induced cytokine responses, but RNA-dependent signaling is unaffected (Ferguson et al., 2012), suggesting selectivity of DNA repair kinases in interacting with nucleic acids in the cytoplasm. In prematurely aged RA T cells, DNA-PKcs triggers JNK activation, mainly causing T cell apoptosis (Shao et al., 2010). RA T cells typically have low expression of ATM, but upregulate DNA-PKcs; possibly a compensatory response. The DNA-PKcs partner proteins Ku70 and Ku80 are diminished in such T cells and DNA damage repair is defective. Both total and phosphorylated DNA-PKcs have been described to be elevated (Shao et al., 2010). Transfection of siRNA specific for DNA-PKcs into DNA-PKcs^{hi} RA T cells corrects their apoptosis susceptibility, suggesting that DNA-PKcs alone is insufficient to rescue the DNA repair defect and that these T cells are able to tolerate DNA breakage only to a certain degree before they undergo cell death (Shao et al., 2010).

In summary, DNA-PKcs and ATM are differentially regulated during T cell aging and fulfill distinct needs. Both kinases partake in nutrient sensing and energy homeostasis, besides their critical role in protecting genome stability. By interacting with cytoplasmic nucleic acids, these kinases aid in the induction of innate immune responses. Whereas ATM^{low} T cells drive tissue inflammation, compensatory upregulation of DNA-PKcs seems not to be tissue protective, suggesting that DNA-PKcs cannot substitute for all ATM functions. DNA-PKcs-dependent cell death per se may be pro-inflammatory, but further studies are needed to decipher the precise mechanisms through which impairment in DNA repair trigger inflammatory reactions in the tissue environment.

MRN complex

To maintain an intact genome, the DDR requires signal sensors, transducers, and effectors. The kinases ATM and ATR function as transducers, able to activate a large number of substrates that eventually drive the repair process or eliminate cells with unrepairable genomes. Unlike other signal transduction pathways that are activated by ligands of receptor kinases, DNA repair kinases are activated by protein complexes that identify DNA structures. The most prominent of such complexes is the MRN complex, composed of the nuclease MRE11A, the ATPase RAD50 and NBS1 mediating protein and protein interaction. The MRN complex specifically detects broken DNA ends, recruits ATM and ATR and orchestrates their function in HR and NHEJ pathways. Downstream of ATM, Chk2-mediated cell-cycle checkpoints arrest cellular division and replication stress elicits Chk1 phosphorylation downstream of ATR (Czornak et al., 2008). It has revealed that the MRN complex also contributes to the activation of ATR pathway and the phosphorylation of Chk1 upon replication stress.

Analysis of humans with mutations in the MRN complex components has been informative in learning how this complex affects cell function, metabolism and pathologies. Hypomorphic mutations of NBS1 result in Nijmegen breakage syndrome (NBS),

characterized by immunodeficiency, microcephaly and increased risk for cancer development, such as lymphoma and leukemia. Circulating T cells from NBS patients show premature immune aging (Chrzanowska et al., 2012), in line with a critical role of NBS1 in immune cell homeostasis. MRE11A hypomorphic mutations lead to an ataxia-telangiectasia-like disorder (A-TLD), a rare autosomal recessive disease resembling A-T and presenting with ataxia and neurodegeneration (Taylor et al., 2004). A single patient has been reported with mutated RAD50, again with a phenotype similar to NBS (Waltes et al., 2009). Cells derived from these patients with mutated MRN components exhibit increased radiosensitivity and impaired checkpoint activation. In mice, null mutations of MRN proteins lead to embryonic lethality, emphasizing the critical contributions in securing genome intactness and cellular survival.

MRE11A is a nuclease with both exonuclease and endonuclease activity, thus able to prepare both single- and double-strand break ends for further repair activity. Knockdown experiments in human T cells, targeting MRE11A, RAD50 and NBS1 with specific siRNA, demonstrate widespread DNA damage foci with the reduction of either molecule, but by far the most damage in MRE11A^{low} cells. Most importantly, MRE11A is positioned at the telomeres, pointing towards a possible role in preparing chromosomal ends and protect them from excessive DNA repair activity. MRE11A protein concentrations proved to be age-dependent and declined with progressive age in both naïve and memory T cell populations. Inhibition of nuclease activity with either pharmacologic or genetic means induced a profile of T cell aging in healthy T cells (CD57⁺, p16 and p21 upregulated) and was a potent trigger for tissue inflammation (Li et al., 2016). MRE11A^{low} T cells displayed a phenotype of aging, upregulating p16, p21 and CD57, but were distinctly low for p53. MRE11A^{low} telomeres had structural damage, including fragility, fusion and complete signal loss. While telomeres in RA T cells have been described to be age-inappropriately shortened, structural abnormalities and not just loss of telomeric sequences seems to be of much more importance.

The precise contribution of MRE11A to stabilizing and capping of healthy telomeres requires further studies. Of interest was the type of tissue inflammation induced by MRE11A^{low} T cells, when adoptively transferred into human synovium-SCID chimeric mice. As in tissue lesions harvested from the inflamed joints of patients with RA, the signature of inflammatory markers included TNF- α , IL-6 and IL-1 β . Remarkably, two anti-inflammatory mediators (TGF- β and IL-10) were markedly diminished, suggesting as one possibility that MRE11A^{low} T cells select the inflammatory infiltrate or undermine physiologic protection mechanisms. The aging phenotype associated with MRE11A reduction was reversible when the nuclease was overexpressed, indicating that dynamic processes control T cell aging and T cell inflammatory potential (Figure 3).

In conclusion, distinct members of the DNA repair machinery have been connected to T cell aging and to downstream T cell effector functions. Where the nuclease MRE11A stands out is in its physical localization to the telomeric ends, in line with a direct role of telomere-replaced proteins in interfering with the aging program. While telomeres have been considered to have clock function in T cell longevity, the more interesting aspect is that telomeric length appears to be of less relevance than telomeric structure. A common denominator of DNA

damage-induced T cell aging is the tendency of such cells to infiltrate into nonlymphoid tissues and elicit an inflammatory lesion. How the reduction in MRE11A or ATM enables T cells to act as a nidus for an inflammatory tissue infiltrate is not completely understood.

Telomeric damage versus telomeric shortening in T cell aging

Telomeres are considered a critical tool in estimating the biologic age of T cell populations (Fujii et al., 2009; Koetz et al., 2000). In mice, telomeric length is correlated with lifespan (Armanios et al., 2009; Blasco et al., 1997; Tomas-Loba et al., 2008). Several genetically modified animal models have been established to further approve the relationship between telomere loss and organismal aging. Reactivation of telomerase sufficiently reverts the aging phenotype observed in the telomerase deficient mice (Jaskelioff et al., 2011). As highly proliferative cells, T cells are exposed to cyclic episodes of intense replication stress. Due to the end replication problem, the length of telomeric sequences shortens with each round of cell division and, consequently, measuring telomeric length has been considered to provide information on the cell's replicative history. Telomeric lengths in memory T cell populations are consistently shorter than in naïve populations (Weng et al., 1998). Notably, telomeres in human T cells essentially never shorten below 5000 kb, thus never reaching telomeric crisis and the contribution of telomeric loss to the induction of T cell aging is therefore unclear. Further complexity is introduced by the expression of telomerase in T cells, a reverse transcriptase that can add sequences to telomeric ends. Only few somatic cells express telomerase, a powerful proto-oncogene present in the vast majority of malignant cells. The premature aging status of RA T cells has been associated with age-inappropriate shortening of telomeres (Koetz et al., 2000), and insufficient expression and activity of telomerase in RA T cells has been reported (Fujii et al., 2009).

While loss of telomeric sequences unequivocally restrains the proliferative capacity and thus clonal expansion of T cells, discussion about the precise mechanisms underlying telomeric attrition have recently been refined. Telomeres in naïve CD4 T cells isolated from RA patients are not only shortened, they are also damaged; raising the important question whether defects in the DNA repair machinery influence telomeric function. Specifically, four damage patterns highly enriched in RA T cells have been identified (Li et al., 2016): apposition of telomeric ends, fusion of chromosomal ends, complete loss of telomeric sequences producing “signal-free” chromosomal ends and telomere fragility, producing broken-off pieces of telomeric DNA. Poor telomeric localization of the nuclease MRE11A has been implicated in rendering T cell telomeres susceptible to injury. These data directly connect the DNA repair machinery to T cell aging (Li et al., 2016).

The current paradigm predicts that repair activity at telomeres, which essentially resemble DNA break sites, is actively repressed to avoid loss of terminal sequences. A protein complex composed of six shelterin proteins covers the telomeric region to protect telomeres from fusion and end nucleolysis (de Lange, 2005). Loss in shelterin proteins results in telomere uncapping and chromosomal fusions. Mutations in shelterins have been found in some human diseases; e.g. aplastic anemia and dyskeratosis congenita. Loss-of-function models have been associated with accelerated aging and declined tissue regenerative capacity (Sarek et al., 2015). The shelterin protein TRF2 specifically interacts with the DNA

damage sensor MRE11A in eukaryotes (Diotti and Loayza, 2011). In *S. cerevisiae*, loss of MRE11A or RAD50 causes telomere shortening (Takata et al., 2005). There is currently no evidence that the packaging of telomeres by shelterin proteins is altered in prematurely aged RA T cells, but detailed studies are lacking. Also, DNA repair proteins, such as ATM, DNA-PKcs and MRE11A, are consistently encountered at the telomeres of healthy T cells, raising the question how they contribute to telomeric maintenance. Studies in healthy human T cells have provided evidence that knockdown of MRE11A not only uncaps telomeres, but also unravels heterochromatin; a phenotype closely associated with arthritogenic potential (Li et al., 2016). How changes in the epigenetic landscape and the structural properties of telomeres translate into pro-inflammatory effector functions is currently not understood. Much effort has gone into objectively measuring T cell age by measuring telomeric lengths. In light of recent insights, it may be more important to assess the structural integrity of telomeres instead of the length of the repetitive nucleotide sequences (Figure 4).

Mitochondrial DNA in aging and inflammatory disease

The interface between metabolism, protecting DNA intactness and dangers imposed by DNA fragments is exemplified in mitochondria. Mitochondria are the cell's power plant, generating much of the energy and the metabolic intermediates that are needed to secure cellular function. Mitochondria contain DNA, the electron transport chain acting as a local source of ROS, their own anti-oxidant protection system and two membrane layers defining membrane gradients and specialized chemical pools. Through their ability to generate ROS and their role as a calcium reservoir, mitochondria control intracellular signaling pathways. Damage to mitochondrial DNA (mtDNA), which encodes for components of the oxidative phosphorylation machinery, jeopardizes energy production and exposes the cell to DNA fragments, often oxidized due to the closeness to the electron transport chain. Maintaining intactness of mtDNA appears particularly important to avoid cellular aging.

mtDNA is detectable in the extracellular space as well as in the cytoplasm, where it functions as a damage-associated molecular pattern (DAMP) to trigger DAMP recognition systems and cause inflammation. The amount of circulating mtDNA is increased in the elderly population and is associated with high levels of inflammatory cytokine production (Pinti et al., 2014). Mutations in mtDNA are believed to disrupt the balance between ROS production and consumption, result in metabolic dysfunction and sustain chronic inflammation (Lagouge and Larsson, 2013; Mohamed Yusoff, 2015). In mice, accumulation of tissue mtDNA promotes apoptosis and correlates with accelerated aging markers. Paradoxically, some mtDNA mutations found in worms that increase ROS production enhance longevity (Alexeyev, 2009). mtDNA has been reported to function as a regulator of innate immunity; essentially acting as a driver of pro-inflammatory responses. By invoking the TLR-9-Myd88 signaling pathway, circulating mtDNA is suspected to contribute to the inflammatory pathogenesis of variable diseases, including RA, atherosclerosis, acute liver injury and hypertension (Fang et al., 2016; Gouloupoulou et al., 2012; Zhang et al., 2014). Once oxidized and released from the mitochondria into the cytoplasm, mtDNA is an endogenous agonist for inflammasome activity, binding to NLRP3, triggering caspase-1 activation and ultimately inducing IL-1 β /IL-18 release (Guo et al., 2015; Shimada et al., 2012).

Besides intercrossing with inflammasome signaling, cytosolic mtDNA also serves as an agonist for type I IFN responses and activates IFN-stimulated gene expression through the GAS-STING axis (Liu et al., 2016). When released from dying neutrophils, mtDNA is a powerful immune regulator and communicates the presence of cell stress in the microenvironment. Recent reports demonstrate the participation of neutrophil extracellular traps (NET) in bacterial clearance and in the induction of sterile inflammatory diseases, such as systemic lupus erythematosus (SLE) (Hakim et al., 2010; Yu and Su, 2013). mtDNA has been identified in NETs, thus indicating the possibility that oxidized extracellular mtDNA regulates type I IFN response and contributes to the pathology observed in patients with SLE. Overall, the degree to which mtDNA is modified by oxidation appears critical to its immunostimulatory potential, directly connecting metabolic activity to immunoregulation. How mtDNA interferes with cellular aging and whether it triggers cellular senescence programs is much less understood. By monitoring how much mtDNA is present and to which degree it is oxidized, the cell has the means to continuously assess mitochondrial stress, metabolic demands, intactness of mtDNA, and thus its own age.

Summary and Conclusions

Inflammation is a critical component of most of the morbidities associated with human aging, delineating the close connection between age-related organ failure and aging of the immune system. As a general rule, immune aging results in deterioration of adaptive immunity and unopposed innate immunity, favoring less specific and less regulated immune responses, compromised tissue regeneration and impaired wound healing. T cells live over many decades and undergo successive cycles of expansion and contraction, imposing high proliferative pressure and, therefore, susceptibility to aging. Proliferatively stressed T cells have adopted multiple strategies to avoid loss of immune homeostasis and preserve host protection against cancer and infection as long as possible. A major stressor in the T cell compartment is the involution of the thymus, minimizing thymic generation of new T cells and enforcing controlled duplication of peripheral T cells; a process named homeostatic proliferation. Successful T cell aging is characterized by the preservation of a high degree of repertoire diversity, avoidance of T cell exhaustion and controlled expansion of the memory compartment (Goronzy and Weyand, 2017). Maladaptive T cell aging encompasses loss of naïve T cells, metabolic reprogramming, accumulation of damaged DNA and telomeres, pronounced membrane ruffling; all promoting tissue-invasive and pro-inflammatory behavior. Maladaptive T cell aging is exemplified in patients with the autoimmune syndrome RA, which have damaged telomeres and age-inappropriate telomeric attrition, display abnormalities in cell cycle control, shift the utilization of glucose away from ATP generation towards synthetic metabolism and form tissue-invasive membrane ruffles.

Based on studies in RA T cells, a molecular taxonomy of T cell aging is emerging (Fig. 1–4). A key defect in RA T cells is the transcriptional repression of the glycolytic enzyme PFKFB3, resulting in slowing of glycolytic flux, less generation of ATP and pyruvate, and less acidification of the extracellular environment. Instead, such pre-aged T cells manufacture NADPH and reduced glutathione due to preferred utilization of the PPP; made possible by upregulation of G6PD. The overall result is an intracellular milieu biased towards reductive elements and biosynthesis of fatty acids, which are deposited as

cytoplasmic lipid droplets. The adaptor molecule TKS5, which connects membrane podosomes with the cytoskeletal machinery, enables T cells to be highly motile and tissue-invasive (Shen et al., 2017). An important component of the aging process in RA T cells is the inability of these cells to secure DNA intactness. With reduced availability of the repair kinase ATM – a consequence of the low ROS environment preventing ATM dimerization – DNA breaks accumulate and the G2/M cell-cycle checkpoint is bypassed. More importantly, lowering of the nuclease MRE11A leads to inefficient telomeric repair, telomeric fragility and heterochromatin unraveling. Notably, genetic and pharmacologic manipulation of glucose metabolism and of DNA repair phenocopy the pro-inflammatory features of RA T cells in healthy T cells and correct the arthritogenic behavior of RA T cells in a humanized mouse model of synovial inflammation.

It is currently unknown whether the T cell aging process in RA patients is simply accelerated and aggravated or whether additional defects in metabolic regulation and DNA repair exist that cause a more severe aging phenotype in patient-derived T cells. Additional unanswered questions include so far unexplored dimensions of cellular aging in RA T cells; specifically, analysis of protein biogenesis, folding, trafficking and degradation; mitochondrial functionality; and fundamental mechanisms of cell-to-cell communication. All of these general domains of cellular function are known to be sensitive to age-related decline. Finally, the interplay of T cell aging and tissue aging is not understood. Attention needs to be given to whether T cells age in sync with other immune cell populations and mesenchymal cells defining the tissue microenvironment, in which inflammatory T cells act. The impact of age-associated shifts in the gut microbiota, now recognized as an instructor of the immune system in the young and the old, will be informative in creating a more comprehensive understanding of organismal aging.

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Abbreviations

EC	endothelial cell
ROS	reactive oxygen species
ATP	adenosine 5'-triphosphate
ER	endoplasmic reticulum
mtDNA	mitochondrial DNA
ETC	electron transport chain
mROS	mitochondrial ROS

NADPH	nicotinamide adenine dinucleotide phosphate
TNF-α	tumor necrosis factor- α
AMPK	AMP-activated protein kinase
VEGF	vascular endothelial growth factor
NF-κB	nuclear factor-kappa B
DDR	DNA damage response
SASP	senescence-associated secretory phenotype
RA	rheumatoid arthritis
PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biophosphatase 3
G6PD	glucose-6-phosphate dehydrogenase
ATM	ataxia telangiectasia mutated
DSB	double-strand breaks
ATR	ATM and RAD3-related
PIKKs	phosphatidylinositol-3-OH-kinase-like kinase
PPP	pentose phosphate pathway
STING	stimulator of interferon genes
DNA-PK	DNA-dependent protein kinase
NHEJ	non-homologous end joining
HR	homologous recombination
SCID	severe combined immunodeficiency
VCAM	vascular cell adhesion molecule
MRE11A	meiotic recombination 11 homolog A
A-TLD	ataxia-telangiectasia-like disorder
NBS	Nijmegen breakage syndrome
TGF	tumor growth factor
DAMP	damage-associated molecular pattern
TLR	toll like receptor
NLRP3	Nod like receptor family pyrin domain containing 3
NET	neutrophil extracellular traps

SLE	systemic lupus erythematosus
TRF2	telomeric repeat-binding factor 2
IFN	interferon
KIR	killer immunoglobulin-like receptors

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Highlights

- T cells from patients with the autoimmune syndrome rheumatoid arthritis (RA) are prematurely aged; providing a model system to define maladaptive aging.
- Aged T cells in RA are metabolically reprogramed; diverging cells towards synthetic and proliferative functions.
- Aged T cells in RA have damaged telomeres due to impaired function of the nuclease MRE11A; eliciting a chronic DNA damage response.
- Aged T cells in RA have reorganized their motility program and overexpress the scaffolding protein Tks5; promoting tissue invasiveness and pro-inflammatory behavior.

Healthy T Cell Aging

Maladaptive T Cell Aging (e.g. in patients with rheumatoid arthritis)

Figure 1. Healthy and Maladaptive T Cell Aging

T cells from patients with the inflammatory syndrome rheumatoid arthritis (RA) age at an accelerated pace. Naïve CD4 T cells from RA patients are metabolically reprogrammed. Due to suppressed glycolysis, they produce less ATP and shunt glucose into the pentose phosphate pathway, yielding high levels of NADPH and low levels of reactive oxygen species (ROS). One outcome is increased lipogenesis, lipid droplet formation, membrane ruffling and accelerated T cell motility. A second outcome is insufficient activation of the DNA repair machinery, affecting the kinase ATM and the nuclease MRE11A. As a consequence, telomeres are uncapped and T cells enter the senescence program. Aged T cells express CD57 and upregulate p16 and p21. Damaged telomeres and membrane podosome formation enable T cells to invade into the synovial tissue and cause chronic synovitis.

RA T cells serve as a model system to explore the aging process in healthy T cells, in which phenotypes are less pronounced but molecular mechanisms may be shared.

Figure 2. Functional Domains affected by low ATM Activity

The serine/threonine kinase Ataxia Telangiectasia Mutated (ATM) belongs to the superfamily of phosphatidylinositol 3-kinase-related kinases and is recognized as an activator of the DNA damage response and a coordinator of cell cycle progression and DNA repair. ATM function is redox sensitive, connecting metabolic activity with the DNA repair machinery. In ATM^{low} cells, mitochondrial oxidative phosphorylation is low, damaged DNA accumulates, and sensing of cytoplasmic DNA fragments is inadequate. Humans born homozygous for mutated ATM age prematurely, are predisposed to cancer and early cardiovascular disease. ATM^{low} T cells in RA patients promote tissue inflammation.

Figure 3. Functional consequences of MRE11A deficiency

MRE11A complexes with RAD50 and NBS1 to form the MRN complex, required for homologous recombination and DNA repair. MRE11A contributes single-strand endonuclease activity and double-strand-specific 3'-5' exonuclease activity. The MRE11A partner RAD50 may be responsible for binding of DNA ends and avoiding end degradation by regulating MRE11's nucleolytic activity. Hypomorphic mutations of MRE11A lead to an ataxia-telangiectasia-like disorder (A-TLD). In human T cells, MRE11A preferentially localizes to telomeric ends, to prevent uncapping and instability and also regulates heterochromatin unraveling. MRE11A^{low} T cells appear to phenocopy ATM^{low} T cells, are hypermobile, tissue-invasive and sustain tissue-damaging inflammation.

Figure 4. Telomere Attrition in T Cell Aging

Telomeric shortening, eliciting a “telomeric crisis”, is recognized as a fundamental mechanism of inducing cellular senescence. However, human T cells maintain telomeric sequences >5,000 kb, and do not enter cellular senescence; raising the question whether loss of telomeric sequences contributes to T cell aging. T cell aging has been associated with telomeric uncapping and insufficient telomeric repair. Specifically, the nuclease MRE11A protects from uncapping and promotes telomeric repair. Theoretically, defects in the function of shelterin proteins could also contribute to T cell aging, but this is insufficiently explored.