Send Orders for Reprints to reprints@benthamscience.ae

Current Aging Science, **2015**, *8*, 131-146

Rheumatoid Arthritis, Immunosenescence and the Hallmarks of Aging

Paulina Chalan[1], Anke van den Berg[2], Bart-Jan Kroesen[3], Liesbeth Brouwer[1] and Annemieke Boots[1]*

[1]*Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands;* [2]*Department of Pathology and Medical Biology, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands;* [3]*Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands*

Abstract: Age is the most important risk factor for the development of infectious diseases, cancer and chronic inflammatory diseases including rheumatoid arthritis (RA). The very act of living causes damage to cells. A network of molecular, cellular and physiological maintenance and repair systems creates a buffering capacity against these damages. Aging leads to progressive shrinkage of the buffering capacity and increases vulnerability. In order to better understand the complex mammalian aging processes, nine hallmarks of aging and their interrelatedness were recently put forward.

RA is a chronic autoimmune disease affecting the joints. Although RA may develop at a young age, the incidence of RA increases with age. It has been suggested that RA may develop as a consequence of premature aging (immunosenescence) of the immune system. Alternatively, premature aging may be the consequence of the inflammatory state in RA. In an effort to answer this chicken and egg conundrum, we here outline and discuss the nine hallmarks of aging, their contribution to the pre-aged phenotype and the effects of treatment on the reversibility of immunosenescence in RA.

Keywords: Aging, immunosenescence, inflammation, rheumatoid arthritis, T-cells.

1. INTRODUCTION

Age is the most important risk factor for development of chronic inflammatory diseases including rheumatoid arthritis (RA). Aging is a complex process, which is not yet fully understood. In order to better understand the complex mammalian aging processes, nine hallmarks of aging and their interrelatedness were recently put forward. In essence, primary hallmarks involve acquisition of cell biological forms of damage such as genomic instability, telomere attrition, epigenetic alterations and loss of proteostasis. Secondary hallmarks involve antagonistic, compensatory responses to these damages such as deregulated nutrient-sensing, mitochondrial dysfunction and cellular senescence. Lastly, the integrative hallmarks represent the result of the primary and secondary events such as stem cell exhaustion and inflammaging, leading to deterioration of cellular and organismal function as seen with aging [1].

Age-associated changes in immune function termed immunosenescence are thought to be responsible for the increased morbidity with age. Both the innate and the adaptive arm of the immune system undergo marked changes with age and contribute to the process of immunosenescence [2]. With age, innate immune mechanisms generally become more active, whereas functioning of the adaptive immune system generally declines.

*Address correspondence to this author at the Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, P.O Box 30.001, 9700 RB, Groningen, The Netherlands; Tel: +31503613747; Fax: +31503619308; E-mail: m.boots@umcg.nl

Immunosenescence is characterized by i) thymic involution leading to a steady decline in the production of naïve T-cells, ii) shrinkage of the T-cell repertoire through continuous antigen stimulation favoring the development of functionally altered, oligoclonal, senescent T-cells identified by CD28 loss and iii) a chronic low degree of inflammation termed inflammaging as evidenced by increased serum levels of inflammatory cytokines such as TNF-α, IL-6 and acute phase proteins [3]. These changes in cellular composition and cellular functions create a pro-inflammatory environment which might accelerate development of RA.

RA is a chronic auto-inflammatory disorder targeting the joints. Although RA can develop in individuals of any age, its incidence continues to increase with age into the seventh decade [4]. When compared to healthy individuals of the same age, RA patients show significantly more prominent features of immune system aging. Despite the growing experimental data, the question whether accelerated immunosenescence is a primary cause of RA or an event secondary to the chronic inflammatory process remains to be answered.

In this review we summarize the experimental evidence for the pre-aged phenotype of different immune cells in RA and their relevance to disease pathogenesis. We also compare features of senescence in peripheral versus local tissues (bone marrow, peripheral blood and the inflamed joint). Furthermore, we outline the contribution of the hallmarks of aging to the pre-aged phenotype in RA. Also, the effects of treatment on the reversibility of immunosenescence in RA

1874-6128/15 $58.00+.00

are discussed. Lastly, novel insights in the molecular pathogenesis of RA relevant to the development of the pre-aged phenotype may guide adequate design of conclusive patient-based research to solve this issue.

2. EVIDENCE FOR A PRE-AGED PHENOTYPE IN RA

2.1. Immunosenescence and T Lymphocytes in RA

Although aging affects all cells of the immune system, T-cells appear to be most sensitive. A prominent feature of T-cell aging is the oligoclonal expansion of CD4+ and especially CD8+ T-cells lacking expression of the co-stimulatory molecule CD28. CD28 co-stimulation, required for efficient T-cell activation and proliferation, is progressively lost with age [3]. Consequences of CD28 loss differ between CD4+ and CD8+ T-cells; in CD8+ T-cells loss of CD28 can lead to dysfunction or towards a regulatory phenotype, whereas in CD4+ T-cells CD28 deficiency is associated with acquisition of novel NK-like functionalities. Loss of CD28 in CD4+ T-cells is associated with an increased production of pro-inflammatory cytokines, increased cytotoxicity, *via* expression of perforin and granzyme B and a propensity to migrate into tissues. Interestingly, CD4+CD28- T-cells were found expanded in patients with several chronic autoimmune conditions, including RA. Expansions were seen in both early and late RA patients and were more prominent in carriers of the RA-susceptibility HLA-DRB1*04 alleles [5]. This suggested that accelerated immunosenescence is a genetically-driven phenomenon that might be causal to RA development rather than the consequence of disease.

Novel functional features of senescent CD28- T-cells have been associated with phenotypical changes, such as *de novo* expression of NK receptors: Immunoglobulin (Ig)-like (*i.e*. killer cell activating receptors [KAR], killer cell inhibitory receptors [KIR]) [6-8] and C-type lectin-like superfamily (NKG2D) receptors [9, 10]. KIRs and KARs are specific for classical MHC class I molecules (HLA-A, HLA-B, HLA-C) and the non-classical MHC molecule HLA-G [11]. Ligands for NKG2D include the stress-induced MHC class I polypeptide-related sequence (MIC) proteins. MIC proteins were found upregulated by RA synoviocytes [10]. Also, CD28- T-cells in RA patients demonstrated increased expression of NK cell-associated receptors CD56 [9, 12, 13] and CD57 [14].

Upregulation of NK receptors likely serves a co-stimulatory role in CD28- T-cells. Cross-linking of KAR in the presence of anti-CD3 induced proliferation of CD4+CD28- T-cells [6]. Engagement of NKG2D, in the presence of anti-CD3, induced proliferation, IFN-γ and TNF-α secretion [10]. Triggering of CD56 alone led to the production of IL-2, TNF-α and MIP-1β [12]. Furthermore, several studies showed upregulation of CD70, a member of the TNF superfamily, by CD28- T-cells [15-17]. CD70 expression lowered the activation threshold of CD28- T-cells [15]. In line with their pro-inflammatory potential, RA patient-derived peripheral blood (PB) CD4+CD28- T-cells [14, 18, 19] and CD4+CD28- T-cell clones [14] produced significantly higher levels of TNF-α and IFN-γ than their CD28+ counterparts.

The exact role of senescent T-cells in the clinical course of RA is still unclear. Numbers of circulating CD4+CD28- T-cells did not correlate with RA clinical parameters such as C-reactive protein (CRP), level of anti-cyclic citrullinated protein antibodies (ACPA), swollen and tender joint counts or disease duration [14, 20, 21]. Some studies demonstrated a correlation between CD4+CD28- T-cells and erosions in RA [22, 23] but this was not confirmed by others [21, 24]. Expansions of CD4+CD28- T-cells in RA have been implicated in the development of atherosclerotic disease [24] and in extra-articular manifestations [21, 24].

The role of senescent T-cells at the level of the joint in RA also remains unclear. Several studies demonstrated that CD4+CD28- T-cells are less frequent in RA synovial fluid (SF) or synovium than in PB [14,18, 20, 25]. This seems to contradict the tissue tracking propensity of CD4+CD28- cells suggested based on the expression of adhesion molecules facilitating tissue infiltration (CD11a, CD49d) [9, 18, 26] and receptors (NKG2D, CX_3CR1) which ligands (MIC, fractalkine) are readily expressed in RA tissue [9, 10]. A possible explanation may involve the restoration of CD28 expression by IL-12 [27] which is abundantly expressed in RA joints [28]. Consistent with this explanation, several similarities between PB CD28- and SF CD28+CD4+ T-cells have been demonstrated. These include the methylation status of IFN-γ promoter, expression of CXCR3, CCR6, CCR7 [19] and clonotypic composition [25].

Several studies highlighted the importance of CX_3CR1, a chemokine receptor exclusively expressed by CD4+CD28- T-cells [9, 29, 30] and its ligand fractalkine (FKN) expressed by fibroblast-like synoviocytes (FLS), as co-stimulatory pair relevant to the local inflammatory process. The CX_3CR1-FKN interaction between CD4+CD28- T-cells and FLS induced expression of pro-inflammatory cytokines and relayed survival signals. Furthermore, the CX_3CR1-FKN interaction facilitated proliferation of FLS [29, 30].

It has been suggested that CD4+CD28- T-cells in RA are autoreactive. So far, conclusive evidence for this notion is lacking. RA-derived CD4+CD28- T-cell clones were shown to proliferate in response to autologous adherent cells *in vitro* [25], while CD4+CD28-NKG2D+ T-cell clones secreted IFN-γ when cultured with autologous MIC+ synoviocytes [10]. CD4+CD28- T-cells did not respond to collagen type II [18], a putative RA-associated autoantigen. In contrast, the reactivity of CD4+CD28- T-cells towards CMV has been clearly demonstrated, and did not differ between RA- and healthy control (HC)-derived or RA- and multiple sclerosis-derived senescent T-cells [18, 31, 32]. Thus, clonally expanded CD4+CD28- T-cells in RA are likely CMV-specific but may also include selfreactive clones [31]. Senescence features reported in various stages of T-cell development in RA are depicted in the Fig. (**1**).

2.2. CMV as Accelerator of T-cell Immunosenescence

CMV infection has been demonstrated to drive immunosenescence directly, by imposing chronic replicative stress and indirectly, by induction of IFN-α expression by plasmacytoid dendritic cells (PDC). IFN-α has been shown to accelerate differentiation and telomere shortening through inhibition of telomerase activity [33].

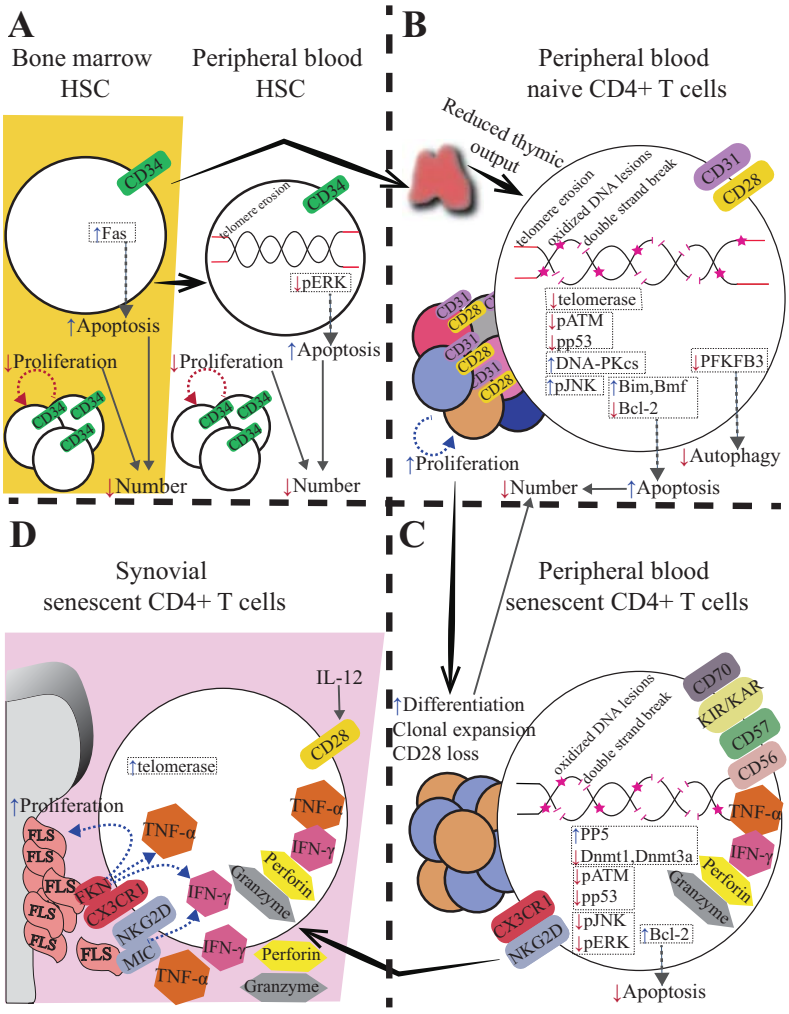

Fig. (1). Depiction of senescence-associated alterations reported within **A**) hematopoietic stem cells (HSC) in bone marrow and peripheral blood, **B**) naive CD4+ T-cells in peripheral blood, **C**) senescent (CD28-) CD4+ T-cells in peripheral blood and **D**) senescent (CD28-) CD4+ T-cells in the inflamed joint of RA patients. **A**) In RA, bone marrow-derived hematopoietic stem cells (defined as CD34+) show impaired proliferative capacity and increased expression of Fas, rendering them apoptosis-sensitive. Consequently, RA patients show reduced HSC numbers at the level of the bone marrow. Peripheral blood-derived CD34+ HSC show similar defects in proliferation and increased susceptibility to apoptosis. Increased apoptosis sensitivity is via impaired ERK pathway signaling and/or age-inappropriate telomere erosion of CD34+ HSC in peripheral blood. **B**) Reduced thymic output in RA was evidenced by reductions of circulating recent thymic emigrants (defined as CD31+ or T-cell receptor excision circle (TREC)+ T-cells). This is explained by inadequate supply of HSC from the bone marrow and/or age-inappropriate enhanced thymic atrophy. Peripheral blood naive T-cells (defined as CD31+CD28+) are characterized by telomere shortening, increased levels of oxidized lesions and double-strand DNA breaks, decreased telomerase activity, deficiency of proteins involved in the DNA damage response (phosphorylated forms of ATM and downstream p53), overactivation of DNA-PKCs-JNK pathway, overexpression of proapoptotic proteins Bim, Bmf and downregulation of antiapoptotic protein Bcl-2. Defects of naive CD4+ T-cells may facilitate their accelerated differentiation towards the senescent state, when subjected to proliferative stress and enhance apoptosis. **C**) Compensatory hyperproliferation of the naive CD4+ T-cell pool leads to the expansion of senescent cells (characterized by the loss of CD28, de novo expression of NK receptors [KIR/KAR, CD56, NKG2D], upregulation of CD57, CD70, CX3CR1, expression of IFN-γ, TNF-α and cytotoxic molecules). Senescent CD4+ T-cells show further increase of DNA damage (double-strand breaks, oxidized DNA lesions and diminished levels of ATM and p53 phosphorylation) relative to naive CD4+ T-cells. Diminished activity of JNK and ERK pathways is associated with the increased expression of anti-apoptotic protein Bcl-2 and concomitant resistance to apoptosis of senescent CD4+ T-cells. **D**) Expression of CX3CR1 facilitates migration of senescent CD4+ T-cells towards soluble Fractalkine (FKN) abundant at the level of synovium. Interaction with FKN expressed on the surface of fibroblast-like synoviocytes (FLS) augments expression of IFN-γ, TNF-α and stimulates release of cytotoxic granules by senescent T-cells and enhances proliferation and FKN expression by FLS. IFN-γ production is also promoted by the interaction of NKG2D with FLS-expressed MIC. IL-12 increased at the level of inflamed joints, may restore CD28 expression by senescent T-cells.

Latent CMV infection has been linked to a pro-inflammatory state as evidenced by increased systemic levels of IL-6 and TNF-α [34]. TNF-α is known to downregulate CD28 expression [35]. Latent CMV infection was found to aggravate the clinical course of RA [36]. CMV-specific CD4+CD28- T-cells were found increased in CMV+ but not CMV- RA patients [20]. These cells represented potent producers of IFN-γ [20, 36, 37].

Despite its role in the development of an immunosenescent phenotype, numerous studies undermine the notion of CMV involvement in RA pathogenesis. The most obvious, epidemiological evidence shows a worldwide CMV prevalence of 40-99%, while only ~1% of all individuals develop RA. A study by Pierer *et al.* which included large cohorts (>200 subjects each) of RA patients and HC, showed similar prevalence of CMV infection as well as comparable titers of anti-CMV antibodies in these groups [36].

Presence of CMV-specific T-cells, CMV DNA or CMV early antigen protein has been demonstrated in RA synovial tissue or synovial fluid but also at inflammatory sites of other autoimmune conditions [38-43].

In conclusion, CMV-driven T-cell immunosenescence is well established in both HC and in RA patients. The data on CD28- T-cells imply that these cells contribute to the inflammatory milieu in several chronic inflammatory conditions including RA. Also, CD28- cells may be involved in (extraarticular) tissue injury. Their contribution to joint pathology is hard to establish, partly because cells may regain CD28 expression *via* an IL-12 dependent mechanism at the level of the joint.

2.3. Immunosenescence and B Lymphocytes in RA

B-cells play an important role in RA pathogenesis based on their ability to produce and secrete autoantibodies and their role as antigen presenting cells [44-47]. Aging has pronounced effects on both numbers and functions of B-cells. Available evidence shows a decline of PB B-cells with age [48]. Yet, peripheral B-cell numbers were not further decreased in newly-diagnosed RA patients when compared to age-matched controls [49].

To the best of our knowledge, no studies have elucidated whether B-cells from RA patients show features of premature senescence. Recently, Rubtsov *et al.* identified a novel population of age-associated B-cells (ABCs), defined as CD19+CD11b+CD11c+, in aged female mice. ABCs were significantly increased in autoimmune-prone mouse strains at the onset of autoimmune disease and were found to be the main source of autoantibody production. The human equivalent of mouse ABCs, defined as CD19+CD11c+CD21- B-cells, were identified in the PB of some elderly female patients with RA [50]. Further studies are required to confirm the accumulation and functional role of ABCs in RA pathogenesis. Besides the increase of potentially pathogenic B-cells, a decrease of IL-10 producing B-cells may contribute to RA development. Both the number and function of immature transitional B-cells (with CD19+$CD24^{high}CD38^{high}$ phenotype) regarded as the main IL-10 producers among B-cells, were found to decrease with age. The frequency of IL-10-expressing cells among $CD24^{high}CD38^{high}$ B-cells was negatively correlated with rheumatoid factor (RF) titers [51]. In conclusion, although it is evident that aging and inflammation affect B-cell numbers and functional subsets, there is no sound evidence to support a role of senescent B-cells in RA pathogenesis.

2.4. Immunosenescence and NK-cells in RA

Aging-associated numerical, phenotypical and functional changes in NK-cells have been comprehensively reviewed [52-55]. Healthy aging is associated with an increase in the number of NK-cells, mainly attributed to the expansion of the differentiated, mature $CD56^{dim}$ subset that develops from the immature $CD56^{bright}$ subset [56]. $CD56^{bright}$ NK-cells were reported to either decrease [57, 58] or remain stable with age [56]. While NK-cell cytotoxicity is well-preserved with age [57], the cytokine and chemokine expression pattern of NK-cells, their proliferative capacity and their responsiveness to cytokines were impaired upon aging [56, 57, 59].

In contrast to healthy elderly, RA patients are characterized by a decline of peripheral NK-cell numbers [60-62]. A decline of these cells may accelerate senescence in RA patients. Support for this notion comes from studies demonstrating that NK-cells clear senescent cells in tumor lesions [63] and eliminate senescent cells involved in tissue damage [64]. These data indicate an immunosurveillant role of NK-cells in RA senescence rather than a pro-inflammatory role.

2.5. Immunosenescence and Monocytes in RA

Three different monocyte subsets can be distinguished based on surface expression of CD14 and CD16 [65, 66], namely the classical monocytes ($CD14^{bright}$ CD16-), the intermediate monocytes ($CD14^{bright}$ CD16+) and the non-classical monocytes ($CD14^{dim}CD16^{bright}$). The latter subset has been suggested to represent senescent monocytes, due to a shorter telomere length compared to classical monocytes and expression of the senescence-associated β-galactosidase (SA-βgal) [67, 68]. The $CD14^{dim}CD16^{bright}$ non-classical monocytes are also more pro-inflammatory and express chemokine receptors facilitating migration to tissues at significantly higher levels than classical monocytes [67, 69]. $CD14^{dim}CD16^{bright}$ monocytes were found to be similarly increased in elderly individuals with atherosclerosis and RA patients when compared to young subjects [67, 70].

Another population of monocytes that was increased with age is the CD56 NK receptor-expressing monocyte subset. These $CD14^{bright}$CD56+ monocytes were found to be potent producers of cytokines and reactive oxygen species (ROS). Interestingly, only young RA patients (<40 years) showed $CD14^{bright}$CD56+ monocyte expansion when compared to HC. As the number of $CD14^{bright}$CD56+ monocytes did not correlate with disease duration, medication or C-reactive protein levels, the cause of the reported increase in these young RA patients remains unclear [71]. More studies are needed to elucidate the nature of senescence-associated changes of monocyte populations and to establish whether these alterations are more profound in RA patients than in aging *per se*.

In conclusion, ample evidence supports a pre-aged phenotype in RA patients within the T-cell compartment, whereas for other PB cells this is less evident. The culprit phenotype is represented by late stage, pro-inflammatory T-cells that have lost CD28 expression. Senescent cells may thrive because NK-cell surveillance is significantly reduced in RA.

3. THE NINE HALLMARKS OF AGING AND RA

Nine common denominators of aging have recently been proposed [1]. The aim of this paragraph is to summarize the

available evidence for the presence of these senescence hallmarks in the context of RA.

3.1. Genomic Instability

Cellular senescence or stable cell cycle arrest can be either telomere-dependent (discussed in "3.2. Telomere shortening") or telomere-independent. The latter develops as a consequence of DNA damage accumulation due to replication errors, ROS, genotoxic drugs or UV light. Excessive DNA damage (including telomere erosion) evokes a persistent DNA damage response (DDR) which induces senescence or programmed cell death, both representing means to prevent malignant transformation. Factors involved in cell fate determination are not known but may include the extent of DNA damage, the strength and duration of DDR signaling and the type of the affected cell [72-74].

Elevated levels of DNA double-strand breaks were demonstrated in PB mononuclear cells (PBMC) [75, 76], or isolated naïve and memory CD4+ T lymphocytes [77, 78] but not neutrophils [79] from RA patients compared to HC. Similarly increased levels of DNA double-strand breaks were seen in naïve CD4+ T-cells from treatment-naïve recently diagnosed RA patients [77]. Also, increased levels of mutagenic DNA adducts such as 8-oxo-guanine (8-oxo-7-hydrodeoxyguanosine or 8-hydroxyguanine) or Heptanone-Etheno-2'-Deoxycytidine (HεdC) were found. 8-oxo-guanine and HεdC represent markers of oxidative DNA damage. In RA, levels of 8-oxo-dG and HεdC were found significantly increased within DNA derived from urine [80], whole blood cells [81], PBMC [75], CD4+ T-cells [77] and naïve CD4+ T-cells [78].

Accumulation of DNA damage in T-cells of RA patients has been associated with defects in DNA repair mechanisms. First evidence came from studies demonstrating an impaired ability of RA patient-derived PBMC to repair the mutagenic base lesion O^6-methylguanine induced by the methylating carcinogen N-methyl-N-nitrosourea [82-84]. Also, a decreased rate of repairing double-strand DNA breaks by RA-derived PBMC has been demonstrated using the DNA unwinding in alkaline solution assay [76]. Studies by Shao *et al.* demonstrated accumulated DNA damage in both naïve and memory T-cells from newly-diagnosed RA patients and related this to decreased levels of the DNA repair kinase ataxia telangiectasia mutated (ATM). ATM deficiency was shown to recapitulate the pre-aged phenotype as seen in RA T-cells whereas overexpression of ATM reconstituted DNA repair capabilities [77, 78]. Thus, both the increase in DNA damage-inducing events such as replicative and oxidative stress as well as the impaired levels of proteins involved in DNA repair are likely the cause of DNA damage accumulation and permanent growth arrest of RA T-cells.

3.2. Telomere Shortening

Telomere erosion is recognized by the cell as a persistent DNA damage [85-87]. Telomere-dependent cell cycle arrest is mediated by upregulation of the p53-dependent DNA damage pathway [88]. Telomeres shorten 50-100 bp after each replication cycle, due to the end-replication problem of DNA polymerase [85, 88]. Activation of telomerase prevents replicative senescence by maintaining telomere function. Telomerase is a ribonucleoprotein complex consisting of the catalytic unit called the telomerase reverse transcriptase (hTERT) and telomerase RNA (TERC). Elongation of telomeres occurs through *de novo* reverse transcription. Expression of hTERT is a limiting factor for telomerase activity. In healthy conditions, telomerase is active only in stem cells and activated T and B lymphocytes [89].

Compared to age-matched HC, RA patients showed enhanced telomere shortening in granulocytes [5], PBMC [90] and CD4+ T-cells [5]. The latter was attributed to telomere erosion of naive, but not memory CD4+ T-cells by Koetz *et al.* [91]. Similarly, telomerase activity following TCR-dependent stimulation was found to be significantly decreased in naive, but not memory, CD4+ T-cells. hTERT deficiency was associated with a reduced proliferative capacity and a higher apoptosis rate of naïve T-cells. In contrast, lymphocytes infiltrating the synovium were characterized by high telomerase activity, indicative of their activated status. The telomerase activity levels of infiltrating cells were correlated with intensity of synovial lining hyperplasia, suggesting active lymphocyte involvement in joint destruction [92]. Interestingly, telomerase activity of anti-CD3 stimulated PBMC was similarly decreased in patients with early RA, multiple sclerosis and patients with flu-like symptoms [93], indicating lack of RA-specificity of telomerase insufficiency.

3.3. Changes in Gene Regulation

Aging is associated with alterations of epigenetic processes. These include mechanisms involved in gene regulation at the transcriptional level, *i.e.* histone modifications (acetylation and methylation), DNA methylation, chromatin remodeling and mechanisms involved in post-transcriptional gene regulation, *i.e.* non-coding (nc)RNAs expression [1]. A growing body of data demonstrates a substantial role of environmental factors in modulation of the epigenome [94]. Low RA concordance rates between monozygotic twins (~15%) [95-98], suggest a high relevance of the interplay between genetics and environmentally-influenced epigenetic alterations in RA pathogenesis.

Epigenetic changes are characteristic of RA synoviocytes. Reported epigenetic alterations include global hypomethylation [99, 100], local hypomethylation of LINE-1 and DR-3 promoters [101, 102], increased HDAC activity [103, 104], Sirt1 overexpression [105] and hyperacetylation [104], local H4 acetylation within MMP-1 promoter [106], sumoylation [107], and distinct pattern of microRNA expression [108].

3.3.1. Histone Modifications

One of the age-associated post-transcriptional (epigenetic) modifications include histone acetylation [1]. Histone acetylation at lysine residues, mediated by histone acetyltransferases (HAT), leads to decreased levels of chromatin condensation, allowing recruitment of the transcriptional machinery and ensuing gene transciption. Inhibition of gene expression, by removal of acetyl groups, is mediated by histone deacetylases (HDAC) [109]. Aging has been associated with a general increase of the histone acetylation status, as evidenced by increased H4K16 acetylation and decreased expression of the class III HDAC (NAD-dependent protein deacetylases sirtuins [Sirt]) [1].

An *in vitro* study employing RA synovial fibroblasts demonstrated a TNF-α-induced increase in HDAC and Sirt1 activity, suggesting that altered histone acetylation may be a feature of inflammation [110, 111]. Moreover, RA patient derived PBMC demonstrated increased HDAC activity [112], but no specific change of Sirt1 activity [105]. The current available data suggest that histone acetylation is differently modulated in aging and RA.

3.3.2. DNA Methylation

Chromatin methylation changes the chromatin structure to a more compact and less easily accessible state for transcription factors. Depending on the gene and cell type, both an age-associated decrease or increase in methylation status may occur. Decreased methylation status leads to enhanced mRNA and protein expression [113, 114]. Methylation occurs at deoxycytosine, primarily within CpG islands (repeated CpG sequences) which are associated with ~70% of the promotors in the vertebrate genome. Age-associated methylation changes have also been found to affect genes lacking CpG islands, *i.e*. CD11a (LFA-1) [115]. Methylation is mediated by DNA methyltransferase Dnmt1, involved in the preservation of the methylation pattern after each cell division, and methyltransferases Dnmt3a and Dnmt3b, which mediate methylation of previously unmethylated DNA [116]. Both Dnmt1 and Dnmt3a expression generally decrease with age [114].

Decreased methylation status has been reported in aging cells of various types, including fibroblasts [117] and stem cells [118]. Hypomethylation of a single CpG in the IL-6 promoter region was found more frequent in RA PBMC when compared to HC PBMC. High levels of IL-6 mRNA in LPS-stimulated macrophages were associated with this single CpG hypomethylation [119]. The most apparent link between hypomethylation and autoimmunity, however, is constituted by the demonstration that functional alterations in senescent CD4+CD28- T-cells are brought about by changes in DNA methylation. Relative to the CD4+CD28+ T-cell population, Dnmt1 and Dnmt3a levels and the DNA methylation status were significantly decreased in senescent CD4+CD28- T-cells. Genes overexpressed in response to hypomethylation included those associated with effector functions of senescent cells, *i.e*. IFN-γ [19] CD70, KIR2DL4 and perforin [16, 17]. Decreased levels of Dnmt1 and Dnmt3 in RA CD4+CD28- T-cells are the consequence of impaired signaling of the ERK and JNK pathways. Similar defects have been noted in CD4+CD28- T-cells generated *in vitro* by repeated stimulation as wells as in CD4+CD28- T-cells from elderly subjects [17]. Interestingly, SF-derived CD4+ T-cells showed prominent hypomethylation of the IFN-γ promoter (IFNG), irrespective of CD28 expression [19].

3.3.3. Chromatin Remodeling

Chromatin remodeling is mediated by enzymes involved in DNA and histone post-transcriptional modifications as well as Heterochromatin Protein 1α (HP1α), Polycomb Group (PcG) or the Nucleosome Remodeling and Deacetylase (NuRD) protein complexes. Their expression levels decrease during aging [1]. RA-derived synovial fibroblasts showed altered levels of the PcG protein EZH2 [120].

3.3.4. ncRNAs

The best studied group of ncRNAs in both health and RA are the microRNAs (miRNAs). These miRNAs are small (~22 nucleotides) single-stranded RNAs that have emerged as important post -transcriptional regulators of gene expression based on limited sequence complementarity.

The aging-associated epigenetic changes influence expression of miRNAs. The role of specific miRNAs in RA has recently been reviewed [121]. Here we will review the miRNAs that link changes in gene regulation, aging and RA. Twenty-four miRNAs have been reported to be involved in gene regulation with aging [122]. MiRNA's may cause aberrant DNA methylation. For example, the miR-29 gene family, miR-143, miR-148a and miR-152 all target Dnmt3a and Dnmt3b. MiRNAs that are implicated in both gene regulation with aging and in RA are miR-16, miR-124a and miR-125a. The expression level of miR-16 was elevated in PBMC of RA patients with active disease and correlated with the erythrocyte sedimentation rate (ESR), CRP and disease activity score 28 (DAS28) [123]. HDAC1, HDAC2 and HDAC3 transcripts are all proven targets of miR-16. It is currently not known if higher miR-16 levels also correspond with lower levels of HDAC transcripts and thus increased histone acetylation in RA patient-derived PBMC. MiR-124a was down regulated in RA synovial fibroblasts [124]. MiR-124a targets the 3` UTR of mRNAs encoding MCP-1 and CDK-2. MiR-124a down regulation thus promotes the production of MCP-1 and CDK-2 proteins by RA synovial fibroblasts. Also, miR-124 (among others) is thought to regulate EZH2, a component of the polycomb repressive complex, involved in chromatin remodeling in various cell types.

Pathways most affected by ageing include genes involved in post transcriptional events such as mRNA splicing [125] Hu antigen R (HuR) is one of the splicing control proteins. HuR is an RNA binding protein which stabilizes mRNA, thereby regulating gene expression [126]. Increased miR-125a levels correlate inversely with HuR in various tumor cells and miR-125a targets the ELAV gene transcript that encodes the HuR protein levels, thereby regulating HuR expression [126]. MiR-125a levels were elevated in plasma from RA patients. These increased plasma levels may reflect the down modulation of HuR expression in PBMC from RA patients [127].

3.4. Loss of Protein Homeostasis

Exogenous or endogenous stressors can lead to unfolding of proteins. Heat shock proteins (hsps) are stress-induced chaperones that refold these proteins or target them for destruction in autophagosomes. The age-associated decline of hsps is associated with reduced longevity [1].

Interestingly, an increased expression of several hsps in RA patient-derived PBMC [128] as well as in RA synovial tissue and fluid has been reported [129-132]. Hsps are upregulated in arthritic joints, likely in response to stress-induced endoplasmic reticulum (ER) hyperreactivity and increased protein turnover. Substantial data support a role for chaperones as autoantigens for T- and B-cells in RA; including detection of hsp-specific autoantibodies [129, 130, 133-

135], presence of autoreactivity-inducing citrulline groups within hsps [136], ability to interact with RA-associated HLA-DRB1*0401 allele [137] and stimulation of T-cell responses [129, 130, 138, 139]. In RA patients, hsps serve as targets of the pathological immune response and their levels do not decline as demonstrated in aging.

Damaged, unfolded or incorrectly folded proteins which cannot be re-folded by hsps, are degraded by cellular proteolytic systems such as the ubiquitin-proteasome and lysosomal systems. This process is impaired with age and leads, in tandem with the decrease of chaperone functions, to accumulation and aggregation of erroneous proteins. Together, this results in an age-associated loss of tissue function [1].

Autophagy is a mechanism that involves degradation of cellular components through the actions of lysosomes. The breakdown of cellular components promotes cellular survival during stress by maintaining cellular energy levels. Autophagy is thus an important process relevant to protein homeostasis, energy metabolism and cell death [140]. Normal aging generally reduces autophagy [1].

The joint environment imposes ER stress to synovial cells due to a high protein turnover [141-143]. Consequently, autophagy is significantly increased in RA synovial fibroblasts. Enhanced autophagy correlated with reduced apoptosis. This led to the assumption that autophagy protects synovial cells from cell death. However, in a recent study using *ex vivo* cultured synovial fibroblasts the authors showed that autophagy may also promote death of RA synovial fibroblasts [144]. Both apoptosis-resistance and apoptosis-induction by autophagy represent means of responding to increased levels of stressors within the inflamed RA joints. Modulation of autophagy in synovial fibroblasts in RA joints was shown to depend on TNF-α [144-146].

Similar to the autophagy-lysosomal system, also elements of the ubiquitin-proteasome system have been found altered in RA synovial fibroblasts [144, 145, 147]. A single nucleotide polymorphism (SNP) mapping to the E3 ubiquitin ligase - *cullin1 (CUL1)* gene *locus,* has been associated with RA susceptibility in the Japanese [148] and north Indian populations [149]. E3 ubiquitin-protein ligase synoviolin, was increased not only in synovial fibroblasts in the joint [150-152], but also within PBMC and serum of RA patients [153]. Synoviolin has been suggested to render synovial cells resistant to apoptosis induced by ER stress [152]. Upregulation of synoviolin *in vitro* by fibroblast-like synoviocytes was TNF-α-dependent [151]. These data suggest that inflammation-mediated ER stress alters proteostasis and that altered proteostasis may enhance inflammation, thereby amplifying the local inflammatory response in RA.

Yang *et al.* reported that RA naïve T-cells are autophagy-deficient. The authors provide evidence that RA T-cells are in an energy-deprived state and thereby rendered apoptosis-sensitive. Increased apoptosis of naive CD4+ T-cells in RA patients may lead to increased homeostatic proliferation and early senescence of the T-cell pool [154]. Thus, metabolic defects may be responsible for loss of proteostasis in RA naïve T cells and may underlie their accelerated senescence.

3.5. Altered Nutrient-Sensing

Pathways involved in nutrient-sensing are thought to have a critical role in aging, as suggested by the overall positive effects of caloric restriction on lifespan. Nutrient-sensing pathways whose dysregulation is consequential for aging include insulin and insulin-like growth factor-1 (IGF-1) signaling (IIS) and the mTOR pathway [1]. Intriguingly, IGF-1 deficiency may shorten or extend lifespan, a paradox which remains unresolved at present. Pituitary-derived growth hormone (GH) induces IGF-1 production in the liver, and IGF-1 suppresses GH in a negative feedback loop [155]. IGF-1 represents one of two ligands of the IGF family, which also consists of six IGF binding proteins (BP). Availability of IGF-1 for the IGF-1 receptor (IGF-1R) is regulated mostly by IGFBP-3 due to its highest abundance in human serum [156]. GH and IGF-1 levels decrease during normal aging [155, 157].

Most studies demonstrated decreased levels of IGF-1 [158-161] and increased levels of IGFBP-3 [156, 158, 162] in serum/plasma of RA patients, which suggests low bioavailability of IGF-1. Acute starvation in RA patients (7 day fasting period) led to a further decline of IGF-1 and reduced measures of inflammation such as ESR, CRP, tender joint count as well as T-cell counts. Interestingly, mitogen activation of CD4+ T-cells after fasting showed increased IL-4 production *in vitro* [163]. These data link nutrient-sensing pathways to altered T-cell activation in RA. However, Matsumoto *et al.* showed that reduced serum levels of IGF-1 in RA did not correlate with clinical parameters of the disease but were negatively correlated with the age of the patients [158]. Moreover, several studies in RA patients failed to detect a change in IGF-1 [164-166] and IGFBP-3 levels [164], and in addition, also observed decreased IGFB-3 levels [160, 166]. In SF, both IGF-1 and IGFBP-3 levels were shown to be increased [167-169]. Thus, the picture is not unequivocal in RA.

Similar contrasting data were obtained for levels of GH [155]. Available data show either decreased [166] or increased [159, 164] GH levels in the periphery of RA patients. A study in newly diagnosed RA patients demonstrated impaired GH production and, similar to IGF-1, a role of pro-inflammatory cytokines (*i.e.* IL-1) in the further suppression of GH [170].

Components of the nutrient-sensing machinery, found to be important in aging, such as Akt (protein kinase B), Foxo and mTOR, have been implicated in the local inflammatory process in RA and were studied mostly in joint-derived FLS [171-176]. In RA circulating T-cells, expression of nutrient-sensing proteins (AMPK and mTOR) was not different compared with controls [154].

In summary, alterations of nutrient-sensing pathways in RA seem to be linked to the increased inflammatory status.

3.6. Mitochondrial Dysfunction

Mitochondrial dysfunction is a feature of aging and may be caused by the accumulation of somatic mutations within the mitochondrial genome (mtDNA), and/or by respiratory chain dysfunction, oxidation of mitochondrial proteins and lipids. Relevant to RA pathogenesis are the induction of

ROS and mtDNA mutations in synovium. The increase of mtDNA mutations were found only when compared to osteoarthritis (OA) control tissue [177], but not when compared to psoriatic arthritis (PsA) tissue [178]. Elevated mtDNA mutation frequency in RA was independent of age. MtDNA mutations positively correlated with macroscopic synovitis, vascularity and with synovial fluid TNF-α and IFN-γ levels. As with other alterations observed within the inflammatory synovial environment, cause-effect relationships are challenging to study. Currently available data suggest involvement of TNF-α, and TNF-α-associated ROS generation in induction of mtDNA mutations [177, 178]. Mitochondrial dysfunction as a result of compromised metabolism induced by the ATP synthase inhibitor oligomycin, has been shown to promote ROS- and NF-κB-dependent induction of inflammatory markers by normal human synoviocytes [179].

Moreover, cell-free mtDNA has been detected in RA synovial fluid and plasma at levels significantly higher than in healthy subjects [180, 181], likely as the result of the increased tissue damage in RA. A study by Collins *et al.* demonstrated that cell-free oxidized mtDNA induces development of arthritis upon its intra-articular injection in mice [181].

Chronic inflammation was found to affect the metabolic competence of T-cells in RA. Their capacity to mobilize aerobic glycolysis for ATP generation was significantly reduced [154].

3.7. Cellular Senescence

Various cell intrinsic and extrinsic stressors can induce cellular senescence. $P16^{ink4a}$ and p53 play major roles in the cellular senescence signaling program [182]. Both proteins are central in the regulation of cell cycling and apoptosis. It has been proposed that the p53 and Rb pathways are responsible for the stress-induced growth arrest, while permanent expression of $p16^{ink4a}$ and $p21^{WAF/CIP1}$ maintain the senescence state [183]. P53 was overexpressed in synovial tissues from both early and late-stage RA when compared to OA and reactive arthritis, while the cells in normal synovial tissue showed significantly less p53 expression [184]. Besides wild-type *p53*, also mutated p53 has been detected in RA synovium [185]. Yamanishi *et al.* analysed *p53* mutations in RA synovium and demonstrated preferential survival of FLS with the mutated *p53* allele. Interestingly, regions with high p53 expression levels also contained high levels of *IL-6* transcripts [185]. This can be explained by the loss of IL-6 suppression dependent on the wild-type *p53*. Induction of *p53* mutations in synovium has been suggested to represent an event secondary to chronic oxidative stress and the continuous need for DNA repair [184-186].

Upregulation of p16 in synovial fibroblasts leads to permanent growth arrest, a feature associated with the senescent phenotype. The *in vitro* upregulation of p16 in macrophages inhibited IL-6 expression [187]. Thus, local induction of p16 expression may have beneficial effects and has been proposed as a possible treatment strategy for RA [188, 189].

Interestingly, downregulation of p53 and other proteins from the DDR pathway in naive CD4+ T-cells has been implicated in T-cell senescence. Conversely, p16 and p53 were highly expressed in CD56+CD28- T-cells [13].

Another marker of cellular senescence is SA-β-gal. SA-β-gal was found to be endogenous lysosomal β -gal [190]. Although SA-β-gal was detected in OA cartilage, we are not aware of any studies reporting on SA-β-gal expression in RA synovium.

3.8. Stem Cell Exhaustion

Several studies suggest that alterations at the level of hematopoietic stem cells (HSC) underlie premature aging of the peripheral T-cell pool in RA.

Cells derived from the bone marrow of RA patients showed less effective colony formation potency when compared to HC-derived bone marrow cells [191-193]. Further studies confirmed the reduced proliferative capacity of CD34+ HSC from RA patients [194-198]. HSC in RA also demonstrated an increased apoptosis rate. Concomitantly, the numbers of CD34+ stem cells were found to be decreased in the bone marrow [194-196] and PB in RA [197].

Colmegna *et al.* [198] demonstrated a dampened ERK signaling pathway in HSC of RA patients. ERK signaling is involved in the growth, differentiation and prevention of apoptosis [199]. More specifically, alteration in RA HSC involved decreased interaction between K-Ras and B-Raf. Ras-induced translocation of Raf to the cellular membrane is necessary for activation of the downstream events of the Raf/MEK/ERK pathway [199]. Defects in K-Ras and B-Raf co-localization (and consequently reduced level of activated pERK) have been linked to senescence-associated impairment of the HSC proliferative response in RA.

Impaired proliferative capacity of HSC in RA has also been associated with decreased telomere length. As HSC are the precursors of T-cells, accelerated telomere shortening in RA HSC may underlie the previously reported premature telomere attrition in RA T-cells [5, 200]. Interestingly, while naive CD4+ T-cells from RA patients were characterized by both telomere erosion and defective telomerase activity, RA HSC demonstrated an increased telomerase activity, suggesting activation of a compensatory pathway [200]. Yet, this did not suffice to prevent telomere erosion in HSC of RA patients carrying the RA-associated HLA-DRB1*04 alleles and led to the hypothesis of genetically-driven premature immunosenescence in RA [5]. Senescence-associated changes within HSC of RA patients were independent from disease duration or disease activity [197] and did not differ between treated and non-treated patients [194, 197]. However, Papadaki *et al.* showed that increased bone marrow levels of TNF-α in RA patients is associated with increased apoptosis and a numerical decrease of CD34+ HSC [194].

3.9. Altered Intercellular Communication Leading to Inflammaging

A low-grade inflammation develops with age, while specific immunity wanes. This persistent inflammation has been termed sterile inflammation due to the lack of a defined pathogenic trigger, or inflammaging. Inflammaging has been associated with functional changes of senescent or terminally

differentiated cells giving rise to the senescence-associated secretory phenotype (SASP) [3].

Both elderly individuals and patients with RA show increased systemic levels of pro-inflammatory cytokines including, among others, IL-6, IL-8 and TNF-α [201]. RA-derived FLS, which undergo replicative senescence induced by serial passage, produce significantly more IL-6, IL-8, vascular endothelial growth factor (VEGF) and prostaglandin E2 (PGE2) in response to IL-1β, when compared to early-passage FLS [202]. Analysis of the molecular mechanisms underlying SASP revealed a critical role of persistent DNA damage response involving increased levels of proteins from the ATM pathway (ATM, NBS1, CHK2) [203, 204]. In contrast, activated p53 may suppress the senescence-associated cytokine (IL-6, IL-8) secretion, while loss of p53 may amplify the SASP [203-205]. Persistent DNA damage has also been suggested as an underlying factor of T-cell replicative senescence in RA. Thus, despite the fact that SASP-inducing ATM and NSB1 were found to be decreased in RA T-cells [77], other proteins involved in the persistent DDR could contribute to SASP in the context of RA. One candidate may involve p53 which was found decreased in RA-derived naive CD4+ T-cells [77].

In conclusion, multiple hallmarks of aging have been identified in early and/or late stage RA patients and are summarized in Table **1**.

4. EFFECTS OF RA TREATMENT ON THE AGED PROFILE IN RA

Biological hallmarks of aging seem to converge in the pre-aged phenotype as seen in RA (Table and Figure). Yet, this premature aging may be inflammation-driven. To gain further insight in cause and consequence, we reviewed the effects of anti-inflammatory treatment on the pre-aged phenotype in RA patients. Several researchers addressed the issue of reversibility of premature T-cell aging upon treatment in RA patients. First, *in vitro* studies demonstrated that chronic TNF-α stimulation-induced CD28 loss by T-cells was partly restored by TNF-α inhibition [210, 211]. Effects of TNF-α blockade on the number of CD28- T-cells in patients, however, are inconclusive. While some studies reported on a reduction of the number of circulating CD4+CD28- T-cells [22, 24], others did not observe any change [37]. Also, anti-TNF-α treatment did not affect the CMV-specific IFN-γ response of CD4+CD28- T-cells [37]. The absolute number of CD4+CD28- T-cells was not different between RA patients receiving abatacept (CTLA-4Ig) or DMARDs [212]. Others demonstrated reduction of CD8+CD28- and late-stage CD8 effector memory, but not CD4+CD28- T-cells after 48 weeks of abatacept treatment. The decreases of CD28- cells among both CD4+ and CD8+ T lymphocyte populations after abatacept therapy were correlated with the clinical response as measured by DAS28/CRP [213]. Interestingly, the absolute numbers of

Table 1. Hallmarks of aging in early and late RA and their modulation upon anti-TNF-α treatment.

	Aging hallmark	Early RA	Reference	Late RA	Reference	Normalization upon anti-TNF-α treatment	Reference
1.	Genomic instability	yes	[77]	yes	[77, 78, 81]	yes	[206, 207]
2.	Loss of telomeres	yes	[91, 93, 200]	yes	[90, 91, 200]	nd	
3.	Gene regulation	nd		yes		nd	
3.1.	Histone modifications	nd		yes	[105, 112]	contradictory results	[112, 208]
3.2.	DNA methylation	nd		yes	[16, 19]	nd	
3.3.	ncRNAs	nd		yes	[123]	nd	
4.	Proteostasis	nd		yes	[128, 130, 154]	nd	
5.	Altered nutrient sensing	yes	[170]	yes	[158-161, 164]	nd	
6.	Mitochondrial dysfunction	nd		yes	[178,180]	yes	[178]
7.	Cellular senescence	yes	[184]	yes	[184]	nd	
8.	Stem cell exhaustion	yes	[194, 197]	yes	[194, 196, 197]	yes	[194, 195, 209]
9.	Inflammaging*	nd		yes	[14, 18, 19]	contradictory results	[22, 24]

1-4 hallmarks of aging, 5-7 antagonistic hallmarks, 8-9 culprit phenotype. nd = not determined.
Hallmarks of aging reflect biological aging but may also be the consequence of inflammation. * The culprit phenotype in RA is represented by pro-inflammatory CD28- T-cells. Pro-inflammatory cytokine production by these cells resembles the SASP.

CD28- T-cells (before treatment) were found to predict the response to abatacept [214].

More importantly, beneficial effects of treatment on premature HSC aging, a putative underlying cause of accelerated T-cell senescence in RA, were reported. The bone marrow niche of RA patients showed decreased apoptosis rates of the CD34+ fraction, increased cell growth potential (assessed by the colony formation assay) and an increased frequency of CD34+ cells after anti-TNF-α treatment [194, 195, 209]. Besides the positive effect on the number of HSC, blocking of TNF-α also resulted in an increase in the levels of recent thymic emigrants, defined as CD31+CD4+ T-cells [209]. Parameters of bone marrow function were not different between RA patients who were or were not on concomitant MTX [196], suggesting that TNF-α- but not MTX-mediated suppression of inflammation, is important for rejuvenation of the HSC pool in RA.

Telomere erosion and reduced telomerase activity in CD4+ T-cells were not affected by treatment of RA patients with MTX or prednisone [91, 200]. Similarly, accelerated telomere erosion in HSC was seen in both treated and not (yet) treated RA patients in cross-sectional analyses only [197]. To the best of our knowledge, the influence of anti-TNF-α agents on telomere length and telomerase activity in RA has not yet been addressed. Neutralization of TNF-α *in vitro* was associated with up regulation of telomerase activity in CD8+ T-cells [215].

Effects of DMARD treatment on cellular senescence, markers of DNA damage and epigenetic alterations relevant to RA were studied only in cell line models. *In vitro*-induced senescence in human fibroblast and cancer cell lines demonstrated a glucocorticoid (GC)-mediated reduction of SASP, such as reduced IL-6 and IL-8 expression. GC did not affect persistent DNA damage, the underlying cause of SASP, nor growth arrest or expression of SA-β-gal [216].

Longitudinal clinical studies demonstrated reduction of the markers of oxidative stress, including 8-hydroxydeoxyguanosine upon treatment with MTX or anti-TNF-α agents. These findings suggest that DMARD treatment reduces ROS-induced DNA damage [206, 207, 217]. Also mtDNA mutations frequency, regarded as a marker of mitochondrial dysfunction, was significantly lower in patients with low disease activity (DAS28 < 3.2) after TNF-α blocking therapy [178].

Studies investigating effects of anti-TNF-α treatment in RA patients on the activity of HAT and HDAC, regulating the acetylation status of histones, yielded contradictory results. HDAC activity, significantly higher in RA at baseline, did not change upon etanercept treatment [112]. Others showed an increase in HAT and a decrease in HDAC activity in a group of RA patients receiving various TNF-α inhibitors and increase of both HAT and HDAC upon RTX treatment [208]. Contradictory results were attributed to the use of different biologicals in these studies.

Thus, the combined evidence suggests that inflammation involving TNF-α, drives premature HSC aging, a putative cause of accelerated T-cell senescence in RA. To further substantiate the notion of reversibility of the senescent phenotype by TNF-α blocking agents or DMARDs, longitudinal clinical studies in well-defined patient cohorts before and after treatment are needed to clarify this issue.

5. PREMATURE AGING IN RA: CAUSE OR CONSEQUENCE?

It has been suggested that premature aging of the immune system may be causal to RA. Telomere shortening of CD4+ T-cells was accelerated in RA patients carrying the RA-associated HLA-DRB1*04 alleles, which led to the hypothesis of genetically-driven premature immunosenescence in RA [5]. However, in light of more recent discoveries, we suggest that the causes of premature senescence in RA may be inflammation-driven. New knowledge of how environmental factors interact with susceptibility genes and the immune system in the development of seropositive RA (ACPA+ and/or RF+) has emerged. ACPA were found to develop more profoundly in individuals carrying the RA-associated HLA-DRB1 shared epitope-containing alleles and can be detected years before symptoms become manifest [218]. Presence of ACPA and RF in pre-RA patients was associated with elevated levels of pro-inflammatory cytokines [219, 220]. ACPA and/or RF may trigger the inflammatory response via an FcγR-mediated mechanism [221, 222]. Thus, the autoantibody-mediated inflammatory state, that precedes RA development by many years, may be responsible for premature immunosenescence in RA.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

In order to evaluate the notion that premature immunosenescence in RA is inflammation-driven, we propose to study immunosenescence features in prospective, longitudinal cohort studies. Inclusion of individuals who are at risk for future RA development (not yet receiving DMARD treatment) would allow to assess the association between inflammatory markers and the immunonesenescent phenotype in the preclinical phase of RA, at disease onset and beyond. Cohorts to be used to assess the preclinical alterations putatively implicated in RA pathogenesis are seropositive arthralgia patients (SAP) and patients with early, undifferentiated arthritis (UA). Both UA and SAP have 30% chance of progressing towards classifiable RA [223, 224]. Furthermore, we propose to study immunesenescence features in late onset seropositive and seronegative RA.

As normal aging is associated with an overall decline of immune function, it will be important to compare healthy age-matched controls, excluding elderly subjects with comorbidities. Assessment of immunosenescence parameters together with disease activity and pro-inflammatory markers at the preclinical stage and at the switch to clinical synovitis, are expected to provide insight into the mechanisms of their mutual regulation.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

PATIENT'S CONSENT

Declared None.

REFERENCES

[1] Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013; 153(6): 1194-217.

[2] Boots AM, Maier AB, Stinissen P, Masson P, Lories RJ, De Keyser F. The influence of ageing on the development and management of rheumatoid arthritis. Nat Rev Rheumatol 2013; 9(10): 604-13.

[3] Weyand CM, Yang Z, Goronzy JJ. T-cell aging in rheumatoid arthritis. Curr Opin Rheumatol 2014; 26(1): 93-100.

[4] Crowson CS, Matteson EL, Myasoedova E, *et al.* The lifetime risk of adult-onset rheumatoid arthritis and other inflammatory autoimmune rheumatic diseases. Arthritis Rheum 2011; 63(3): 633-9.

[5] Schonland SO, Lopez C, Widmann T, *et al.* Premature telomeric loss in rheumatoid arthritis is genetically determined and involves both myeloid and lymphoid cell lineages. Proc Natl Acad Sci USA 2003; 100(23): 13471-6.

[6] Namekawa T, Snyder MR, Yen JH, *et al.* Killer cell activating receptors function as costimulatory molecules on CD4+CD28null T cells clonally expanded in rheumatoid arthritis. J Immunol 2000; 165(2): 1138-45.

[7] Warrington KJ, Takemura S, Goronzy JJ, Weyand CM. CD4+, CD28- T cells in rheumatoid arthritis patients combine features of the innate and adaptive immune systems. Arthritis Rheum 2001; 44(1): 13-20.

[8] Snyder MR, Muegge LO, Offord C, *et al.* Formation of the killer Ig-like receptor repertoire on CD4+CD28null T cells. J Immunol 2002; 168(8): 3839-46.

[9] Broux B, Markovic-Plese S, Stinissen P, Hellings N. Pathogenic features of CD4+CD28- T cells in immune disorders. Trends Mol Med; 18(8): 446-53.

[10] Groh V, Bruhl A, El-Gabalawy H, Nelson JL, Spies T. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. Proc Natl Acad Sci USA 2003; 100(16): 9452-7.

[11] Li Y, Mariuzza RA. Structural basis for recognition of cellular and viral ligands by NK cell receptors. Front Immunol 2014; 5: 123.

[12] Michel JJ, Turesson C, Lemster B, *et al.* CD56-expressing T cells that have features of senescence are expanded in rheumatoid arthritis. Arthritis Rheum 2007; 56(1): 43-57.

[13] Lemster BH, Michel JJ, Montag DT, *et al.* Induction of CD56 and TCR-independent activation of T cells with aging. J Immunol 2008; 180(3): 1979-90.

[14] Fasth AE, Cao D, Van Vollenhoven R, Trollmo C, Malmstrom V. CD28nullCD4+ T cells--characterization of an effector memory T-cell population in patients with rheumatoid arthritis. Scand J Immunol; 60(1-2): 199-208.

[15] Lee WW, Yang ZZ, Li G, Weyand CM, Goronzy JJ. Unchecked CD70 expression on T cells lowers threshold for T cell activation in rheumatoid arthritis. J Immunol 2007; 179(4): 2609-15.

[16] Liu Y, Chen Y, Richardson B. Decreased DNA methyltransferase levels contribute to abnormal gene expression in "senescent" CD4(+)CD28(-) T cells. Clin Immunol 2009; 132(2): 257-65.

[17] Chen Y, Gorelik GJ, Strickland FM, Richardson BC. Decreased ERK and JNK signaling contribute to gene overexpression in "senescent" CD4+CD28- T cells through epigenetic mechanisms. J Leukoc Biol 2010; 87(1): 137-45.

[18] Thewissen M, Somers V, Hellings N, Fraussen J, Damoiseaux J, Stinissen P. CD4+CD28null T cells in autoimmune disease: pathogenic features and decreased susceptibility to immunoregulation. J Immunol 2007; 179(10): 6514-23.

[19] Pieper J, Johansson S, Snir O, *et al.* Peripheral and site-specific CD4(+) CD28(null) T cells from rheumatoid arthritis patients show distinct characteristics. Scand J Immunol 2014; 79(2): 149-55.

[20] Fasth AE, Snir O, Johansson AA, *et al.* Skewed distribution of proinflammatory CD4+CD28null T cells in rheumatoid arthritis. Arthritis Res Ther 2007; 9(5): R87.

[21] Martens PB, Goronzy JJ, Schaid D, Weyand CM. Expansion of unusual CD4+ T cells in severe rheumatoid arthritis. Arthritis Rheum 1997; 40(6): 1106-14.

[22] Pawlik A, Ostanek L, Brzosko I, *et al.* The expansion of CD4+CD28- T cells in patients with rheumatoid arthritis. Arthritis Res Ther 2003; 5(4): R210-3.

[23] Goronzy JJ, Matteson EL, Fulbright JW, *et al.* Prognostic markers of radiographic progression in early rheumatoid arthritis. Arthritis Rheum 2004; 50(1): 43-54.

[24] Gerli R, Schillaci G, Giordano A, *et al.* CD4+CD28- T lymphocytes contribute to early atherosclerotic damage in rheumatoid arthritis patients. Circulation 2004; 109(22): 2744-8.

[25] Schmidt D, Goronzy JJ, Weyand CM. CD4+ CD7- CD28- T cells are expanded in rheumatoid arthritis and are characterized by autoreactivity. J Clin Invest 1996; 97(9): 2027-37.

[26] Namekawa T, Wagner UG, Goronzy JJ, Weyand CM. Functional subsets of CD4 T cells in rheumatoid synovitis. Arthritis Rheum 1998; 41(12): 2108-16.

[27] Warrington KJ, Vallejo AN, Weyand CM, Goronzy JJ. CD28 loss in senescent CD4+ T cells: reversal by interleukin-12 stimulation. Blood 2003; 101(9): 3543-9.

[28] Kim W, Min S, Cho M, *et al.* The role of IL-12 in inflammatory activity of patients with rheumatoid arthritis (RA). Clin Exp Immunol 2000; 119(1): 175-81.

[29] Sawai H, Park YW, Roberson J, Imai T, Goronzy JJ, Weyand CM. T cell costimulation by fractalkine-expressing synoviocytes in rheumatoid arthritis. Arthritis Rheum 2005; 52(5): 1392-401.

[30] Sawai H, Park YW, He X, Goronzy JJ, Weyand CM. Fractalkine mediates T cell-dependent proliferation of synovial fibroblasts in rheumatoid arthritis. Arthritis Rheum 2007; 56(10): 3215-25.

[31] Thewissen M, Somers V, Venken K, *et al.* Analyses of immunosenescent markers in patients with autoimmune disease. Clin Immunol 2007; 123(2): 209-18.

[32] Van Bergen J, Kooy-Winkelaar EM, Van Dongen H, *et al.* Functional killer Ig-like receptors on human memory CD4+ T cells specific for cytomegalovirus. J Immunol 2009; 182(7): 4175-82.

[33] Fletcher JM, Vukmanovic-Stejic M, Dunne PJ, *et al.* Cytomegalovirus-specific CD4+ T cells in healthy carriers are continuously driven to replicative exhaustion. J Immunol 2005; 175(12): 8218-25.

[34] Pawelec G, McElhaney JE, Aiello AE, Derhovanessian E. The impact of CMV infection on survival in older humans. Curr Opin Immunol 2012; 24(4): 507-11.

[35] Lewis DE, Merched-Sauvage M, Goronzy JJ, Weyand CM, Vallejo AN. Tumor necrosis factor-alpha and CD80 modulate CD28 expression through a similar mechanism of T-cell receptor-independent inhibition of transcription. J Biol Chem 2004; 279(28): 29130-8.

[36] Pierer M, Rothe K, Quandt D, *et al.* Association of anticytomegalovirus seropositivity with more severe joint destruction and more frequent joint surgery in rheumatoid arthritis. Arthritis Rheum 2012; 64(6): 1740-9.

[37] Davignon JL, Boyer JF, Jamard B, Nigon D, Constantin A, Cantagrel A. Maintenance of cytomegalovirus-specific CD4pos T-cell response in rheumatoid arthritis patients receiving anti-tumor necrosis factor treatments. Arthritis Res Ther 2010; 12(4): R142.

[38] Murayama T, Jisaki F, Ayata M, *et al.* Cytomegalovirus genomes demonstrated by polymerase chain reaction in synovial fluid from rheumatoid arthritis patients. Clin Exp Rheumatol 1992 Mar-Apr; 10(2): 161-4.

[39] Tamm A, Ziegler T, Lautenschlager I, *et al.* Detection of cytomegalovirus DNA in cells from synovial fluid and peripheral blood of patients with early rheumatoid arthritis. J Rheumatol 1993; 20(9): 1489-93.

[40] Scotet E, Peyrat MA, Saulquin X, *et al.* Frequent enrichment for CD8 T cells reactive against common herpes viruses in chronic inflammatory lesions: towards a reassessment of the physiopathological significance of T cell clonal expansions found in autoimmune inflammatory processes. Eur J Immunol 1999; 29(3): 973-85.

[41] Edinger JW, Bonneville M, Scotet E, Houssaint E, Schumacher HR, Posnett DN. EBV gene expression not altered in rheumatoid

synovia despite the presence of EBV antigen-specific T cell clones. J Immunol 1999; 162(6): 3694-701.

[42] Stahl HD, Hubner B, Seidl B, *et al.* Detection of multiple viral DNA species in synovial tissue and fluid of patients with early arthritis. Ann Rheum Dis 2000; 59(5): 342-6.

[43] Mehraein Y, Lennerz C, Ehlhardt S, Remberger K, Ojak A, Zang KD. Latent Epstein-Barr virus (EBV) infection and cytomegalovirus (CMV) infection in synovial tissue of autoimmune chronic arthritis determined by RNA- and DNA-in situ hybridization. Mod Pathol 2004; 17(7): 781-9.

[44] Moura RA, Weinmann P, Pereira PA, *et al.* Alterations on peripheral blood B-cell subpopulations in very early arthritis patients. Rheumatology (Oxford) 2010; 49(6): 1082-92.

[45] Moura RA, Cascao R, Perpetuo I, *et al.* Cytokine pattern in very early rheumatoid arthritis favours B-cell activation and survival. Rheumatology (Oxford) 2011; 50(2): 278-82.

[46] Bosello S, Youinou P, Daridon C, *et al.* Concentrations of BAFF correlate with autoantibody levels, clinical disease activity, and response to treatment in early rheumatoid arthritis. J Rheumatol 2008; 35(7): 1256-64.

[47] Gottenberg JE, Miceli-Richard C, Ducot B, Goupille P, Combe B, Mariette X. Markers of B-lymphocyte activation are elevated in patients with early rheumatoid arthritis and correlated with disease activity in the ESPOIR cohort. Arthritis Res Ther 2009; 11(4): R114.

[48] Morbach H, Eichhorn EM, Liese JG, Girschick HJ. Reference values for B cell subpopulations from infancy to adulthood. Clin Exp Immunol 2010; 162(2): 271-9.

[49] van der Geest KS, Abdulahad WH, Chalan P, *et al.* Disturbed B cell homeostasis in newly diagnosed giant cell arteritis and polymyalgia rheumatica. Arthritis Rheumatol 2014; 66(7): 1927-38.

[50] Rubtsov AV, Rubtsova K, Fischer A, *et al.* Toll-like receptor 7 (TLR7)-driven accumulation of a novel CD11c(+) B-cell population is important for the development of autoimmunity. Blood 2011; 118(5): 1305-15.

[51] Duggal NA, Upton J, Phillips AC, Sapey E, Lord JM. An age-related numerical and functional deficit in CD19(+) CD24(hi) CD38(hi) B cells is associated with an increase in systemic autoimmunity. Aging Cell 2013; 12(5): 873-81.

[52] Gayoso I, Sanchez-Correa B, Campos C, *et al.* Immunosenescence of human natural killer cells. J Innate Immun 2011; 3(4): 337-43.

[53] Mariani E, Meneghetti A, Formentini I, *et al.* Telomere length and telomerase activity: effect of ageing on human NK cells. Mech Ageing Dev 2003; 124(4): 403-8.

[54] Mariani E, Meneghetti A, Formentini I, *et al.* Different rates of telomere shortening and telomerase activity reduction in CD8 T and CD16 NK lymphocytes with ageing. Exp Gerontol 2003; 38(6): 653-9.

[55] Ouyang Q, Baerlocher G, Vulto I, Lansdorp PM. Telomere length in human natural killer cell subsets. Ann N Y Acad Sci 2007; 1106: 240-52.

[56] Borrego F, Alonso MC, Galiani MD, *et al.* NK phenotypic markers and IL2 response in NK cells from elderly people. Exp Gerontol 1999; 34(2): 253-65.

[57] Krishnaraj R. Senescence and cytokines modulate the NK cell expression. Mech Ageing Dev 1997; 96(1-3): 89-101.

[58] Chidrawar SM, Khan N, Chan YL, Nayak L, Moss PA. Ageing is associated with a decline in peripheral blood CD56bright NK cells. Immun Ageing 2006; 3: 10.

[59] Solana R, Mariani E. NK and NK/T cells in human senescence. Vaccine 2000; 18(16): 1613-20.

[60] Shibatomi K, Ida H, Yamasaki S, *et al.* A novel role for interleukin-18 in human natural killer cell death: high serum levels and low natural killer cell numbers in patients with systemic autoimmune diseases. Arthritis Rheum 2001; 44(4): 884-92.

[61] Aggarwal A, Sharma A, Bhatnagar A. Role of cytolytic impairment of natural killer and natural killer T-cell populations in rheumatoid arthritis. Clin Rheumatol 2014; 33(8): 1067-78.

[62] Conigliaro P, Triggianese P, Perricone C, *et al.* Restoration of peripheral blood natural killer and B cell levels in patients affected by rheumatoid and psoriatic arthritis during etanercept treatment. Clin Exp Immunol 2014; 177(1): 234-43.

[63] Perez-Mancera PA, Young AR, Narita M. Inside and out: the activities of senescence in cancer. Nat Rev Cancer 2014; 14(8): 547-58.

[64] Sagiv A, Krizhanovsky V. Immunosurveillance of senescent cells: the bright side of the senescence program. Biogerontology 2013; 14(6): 617-28.

[65] Ziegler-Heitbrock HW, Fingerle G, Strobel M, *et al.* The novel subset of CD14+/CD16+ blood monocytes exhibits features of tissue macrophages. Eur J Immunol 1993; 23(9): 2053-8.

[66] Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: their role in infection and inflammation. J Leukoc Biol 2007; 81(3): 584-92.

[67] Merino A, Buendia P, Martin-Malo A, Aljama P, Ramirez R, Carracedo J. Senescent CD14+CD16+ monocytes exhibit proinflammatory and proatherosclerotic activity. J Immunol 2011; 186(3): 1809-15.

[68] Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O. Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and *in vivo*. Nat Protoc 2009; 4(12): 1798-806.

[69] Kawanaka N, Yamamura M, Aita T, *et al.* CD14+,CD16+ blood monocytes and joint inflammation in rheumatoid arthritis. Arthritis Rheum 2002; 46(10): 2578-86.

[70] Seidler S, Zimmermann HW, Bartneck M, Trautwein C, Tacke F. Age-dependent alterations of monocyte subsets and monocyte-related chemokine pathways in healthy adults. BMC Immunol 2010; 11: 30-2172-11-30.

[71] Krasselt M, Baerwald C, Wagner U, Rossol M. CD56+ monocytes have a dysregulated cytokine response to lipopolysaccharide and accumulate in rheumatoid arthritis and immunosenescence. Arthritis Res Ther 2013; 15(5): R139.

[72] Naylor RM, Baker DJ, van Deursen JM. Senescent cells: a novel therapeutic target for aging and age-related diseases. Clin Pharmacol Ther 2013; 93(1): 105-16.

[73] d'Adda di Fagagna F. Living on a break: cellular senescence as a DNA-damage response. Nat Rev Cancer 2008; 8(7): 512-22.

[74] Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. Genes Dev 2010; 24(22): 2463-79.

[75] Bashir S, Harris G, Denman MA, Blake DR, Winyard PG. Oxidative DNA damage and cellular sensitivity to oxidative stress in human autoimmune diseases. Ann Rheum Dis 1993; 52(9): 659-66.

[76] Bhusate LL, Herbert KE, Scott DL, Perrett D. Increased DNA strand breaks in mononuclear cells from patients with rheumatoid arthritis. Ann Rheum Dis 1992; 51(1): 8-12.

[77] Shao L, Fujii H, Colmegna I, Oishi H, Goronzy JJ, Weyand CM. Deficiency of the DNA repair enzyme ATM in rheumatoid arthritis. J Exp Med 2009; 206(6): 1435-49.

[78] Shao L, Goronzy JJ, Weyand CM. DNA-dependent protein kinase catalytic subunit mediates T-cell loss in rheumatoid arthritis. EMBO Mol Med 2010; 2(10): 415-27.

[79] McConnell JR, Crockard AD, Cairns AP, Bell AL. Neutrophils from systemic lupus erythematosus patients demonstrate increased nuclear DNA damage. Clin Exp Rheumatol 2002; 20(5): 653-60.

[80] Jikimoto T, Nishikubo Y, Koshiba M, *et al.* Thioredoxin as a biomarker for oxidative stress in patients with rheumatoid arthritis. Mol Immunol 2002; 38(10): 765-72.

[81] Ogawa M, Matsuda T, Ogata A, *et al.* DNA damage in rheumatoid arthritis: an age-dependent increase in the lipid peroxidation-derived DNA adduct, heptanone-etheno-2'-deoxycytidine. Autoimmune Dis 2013; 2013: 183-487.

[82] Harris G, Asbery L, Lawley PD, Denman AM, Hylton W. Defective repair of 0(6)-methylguanine in autoimmune diseases. Lancet 1982; 2(8305): 952-6.

[83] Lawley PD, Topper R, Denman AM, Hylton W, Hill ID, Harris G. Increased sensitivity of lymphocytes from patients with systemic autoimmune diseases to DNA alkylation by the methylating carcinogen N-methyl-N-nitrosourea. Ann Rheum Dis 1988; 47(6): 445-51.

[84] Colaco CB, Harris G, Lawley PD, Lydyard PM, Roitt IM. Deficient repair of O6-methylguanine in lymphocytes from rheumatoid arthritis families may be an acquired defect. Clin Exp Immunol 1988; 72(1): 15-19.

[85] Djojosubroto MW, Choi YS, Lee HW, Rudolph KL. Telomeres and telomerase in aging, regeneration and cancer. Mol Cells 2003; 15(2): 164-75.

[86] Beliveau A, Bassett E, Lo AT, *et al.* P53-Dependent Integration of Telomere and Growth Factor Deprivation Signals. Proc Natl Acad Sci USA 2007; 104(11): 4431-6.

[87] Mason PJ, Perdigones N. Telomere biology and translational research. Transl Res 2013; 162(6): 333-42.

[88] Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). Mol Cell 2004; 14(4): 501-13.

[89] Hodes RJ, Hathcock KS, Weng NP. Telomeres in T and B cells. Nat Rev Immunol 2002; 2(9): 699-706.

[90] Pedersen-Lane JH, Zurier RB, Lawrence DA. Analysis of the thiol status of peripheral blood leukocytes in rheumatoid arthritis patients. J Leukoc Biol 2007; 81(4): 934-41.

[91] Koetz K, Bryl E, Spickschen K, O'Fallon WM, Goronzy JJ, Weyand CM. T cell homeostasis in patients with rheumatoid arthritis. Proc Natl Acad Sci USA 2000; 97(16): 9203-8.

[92] Yudoh K, Matsuno H, Nezuka T, Kimura T. Different mechanisms of synovial hyperplasia in rheumatoid arthritis and pigmented villonodular synovitis: the role of telomerase activity in synovial proliferation. Arthritis Rheum 1999; 42(4): 669-77.

[93] Thewissen M, Linsen L, Geusens P, Raus J, Stinissen P. Impaired activation-induced telomerase activity in PBMC of early but not chronic rheumatoid arthritis patients. Immunol Lett 2005; 100(2): 205-10.

[94] Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. Nat Rev Genet 2012; 13(2): 97-109.

[95] Meda F, Folci M, Baccarelli A, Selmi C. The epigenetics of autoimmunity. Cell Mol Immunol 2011; 8(3): 226-36.

[96] Ballestar E. Epigenetic alterations in autoimmune rheumatic diseases. Nat Rev Rheumatol 2011; 7(5): 263-71.

[97] Zufferey F, Williams FM, Spector TD. Epigenetics and methylation in the rheumatic diseases. Semin Arthritis Rheum 2014; 43(5): 692-700.

[98] Firestein GS. The disease formerly known as rheumatoid arthritis. Arthritis Res Ther 2014; 16(3): 114.

[99] de la Rica L, Urquiza JM, Gomez-Cabrero D, *et al.* Identification of novel markers in rheumatoid arthritis through integrated analysis of DNA methylation and microRNA expression. J Autoimmun 2013; 41: 6-16.

[100] Karouzakis E, Gay RE, Michel BA, Gay S, Neidhart M. DNA hypomethylation in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 2009; 60(12): 3613-22.

[101] Neidhart M, Rethage J, Kuchen S, *et al.* Retrotransposable L1 elements expressed in rheumatoid arthritis synovial tissue: association with genomic DNA hypomethylation and influence on gene expression. Arthritis Rheum 2000; 43(12): 2634-47.

[102] Takami N, Osawa K, Miura Y, *et al.* Hypermethylated promoter region of DR3, the death receptor 3 gene, in rheumatoid arthritis synovial cells. Arthritis Rheum 2006; 54(3): 779-87.

[103] Grabiec AM, Reedquist KA. Histone deacetylases in RA: epigenetics and epiphenomena. Arthritis Res Ther 2010; 12(5): 142.

[104] Huber LC, Brock M, Hemmatazad H, *et al.* Histone deacetylase/acetylase activity in total synovial tissue derived from rheumatoid arthritis and osteoarthritis patients. Arthritis Rheum 2007; 56(4): 1087-93.

[105] Wendling D, Vidon C, Abbas W, Guillot X, Toussirot E, Herbein G. Sirt1 activity in peripheral blood mononuclear cells from patients with rheumatoid arthritis. Joint Bone Spine 2014; 81(5): 462-3.

[106] Maciejewska-Rodrigues H, Karouzakis E, Strietholt S, *et al.* Epigenetics and rheumatoid arthritis: the role of SENP1 in the regulation of MMP-1 expression. J Autoimmun 2010; 35(1): 15-22.

[107] Meinecke I, Cinski A, Baier A, *et al.* Modification of nuclear PML protein by SUMO-1 regulates Fas-induced apoptosis in rheumatoid arthritis synovial fibroblasts. Proc Natl Acad Sci USA 2007; 104(12): 5073-8.

[108] Stanczyk J, Pedrioli DM, Brentano F, *et al.* Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. Arthritis Rheum 2008; 58(4): 1001-9.

[109] Grabiec AM, Reedquist KA. The ascent of acetylation in the epigenetics of rheumatoid arthritis. Nat Rev Rheumatol 2013; 9(5): 311-8.

[110] Kawabata T, Nishida K, Takasugi K, *et al.* Increased activity and expression of histone deacetylase 1 in relation to tumor necrosis factor-alpha in synovial tissue of rheumatoid arthritis. Arthritis Res Ther 2010; 12(4): R133.

[111] Niederer F, Ospelt C, Brentano F, *et al.* SIRT1 overexpression in the rheumatoid arthritis synovium contributes to proinflammatory cytokine production and apoptosis resistance. Ann Rheum Dis 2011; 70(10): 1866-73.

[112] Gillespie J, Savic S, Wong C, *et al.* Histone deacetylases are dysregulated in rheumatoid arthritis and a novel histone deacetylase 3-selective inhibitor reduces interleukin-6 production by peripheral blood mononuclear cells from rheumatoid arthritis patients. Arthritis Rheum 2012; 64(2): 418-22.

[113] Richardson B. DNA methylation and autoimmune disease. Clin Immunol 2003; 109(1): 72-9.

[114] Richardson B. Impact of aging on DNA methylation. Ageing Res Rev 2003; 2(3): 245-61.

[115] Deaton AM, Bird A. CpG islands and the regulation of transcription. Genes Dev 2011; 25(10): 1010-22.

[116] Attwood JT, Yung RL, Richardson BC. DNA methylation and the regulation of gene transcription. Cell Mol Life Sci 2002; 59(2): 241-57.

[117] Lopatina N, Haskell JF, Andrews LG, Poole JC, Saldanha S, Tollefsbol T. Differential maintenance and de novo methylating activity by three DNA methyltransferases in aging and immortalized fibroblasts. J Cell Biochem 2002; 84(2): 324-34.

[118] So AY, Jung JW, Lee S, Kim HS, Kang KS. DNA methyltransferase controls stem cell aging by regulating BMI1 and EZH2 through microRNAs. PLoS One 2011; 6(5): e19503.

[119] Nile CJ, Read RC, Akil M, Duff GW, Wilson AG. Methylation status of a single CpG site in the IL6 promoter is related to IL6 messenger RNA levels and rheumatoid arthritis. Arthritis Rheum 2008; 58(9): 2686-93.

[120] Trenkmann M, Brock M, Gay RE, *et al.* Expression and function of EZH2 in synovial fibroblasts: epigenetic repression of the Wnt inhibitor SFRP1 in rheumatoid arthritis. Ann Rheum Dis 2011; 70(8): 1482-8.

[121] Miao CG, Yang YY, He X, *et al.* New advances of microRNAs in the pathogenesis of rheumatoid arthritis, with a focus on the crosstalk between DNA methylation and the microRNA machinery. Cell Signal 2013; 25(5): 1118-25.

[122] Harries LW. MicroRNAs as Mediators of the Ageing Process. Genes (Basel) 2014; 5(3): 656-70.

[123] Feng ZT, Li J, Ren J, Lv Z. Expression of miR-146a and miR-16 in peripheral blood mononuclear cells of patients with rheumatoid arthritis and their correlation to the disease activity. Nan Fang Yi Ke Da Xue Xue Bao 2011; 31(2): 320-3.

[124] Nakamachi Y, Kawano S, Takenokuchi M, *et al.* MicroRNA-124a is a key regulator of proliferation and monocyte chemoattractant protein 1 secretion in fibroblast-like synoviocytes from patients with rheumatoid arthritis. Arthritis Rheum 2009; 60(5): 1294-304.

[125] Harries LW, Hernandez D, Henley W, *et al.* Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. Aging Cell 2011; 10(5): 868-78.

[126] van Kouwenhove M, Kedde M, Agami R. MicroRNA regulation by RNA-binding proteins and its implications for cancer. Nat Rev Cancer 2011; 11(9): 644-56.

[127] Sugihara M, Tsutsumi A, Suzuki E, *et al.* Effects of infliximab therapy on gene expression levels of tumor necrosis factor alpha, tristetraprolin, T cell intracellular antigen 1, and Hu antigen R in patients with rheumatoid arthritis. Arthritis Rheum 2007; 56(7): 2160-69.

[128] Edwards CJ, Feldman JL, Beech J, *et al.* Molecular profile of peripheral blood mononuclear cells from patients with rheumatoid arthritis. Mol Med 2007 Jan-Feb; 13(1-2): 40-58.

[129] Blass S, Union A, Raymackers J, *et al.* The stress protein BiP is overexpressed and is a major B and T cell target in rheumatoid arthritis. Arthritis Rheum 2001; 44(4): 761-71.

[130] Corrigall VM, Bodman-Smith MD, Fife MS, *et al.* The human endoplasmic reticulum molecular chaperone BiP is an autoantigen for rheumatoid arthritis and prevents the induction of experimental arthritis. J Immunol 2001; 166(3): 1492-98.

[131] Martin CA, Carsons SE, Kowalewski R, Bernstein D, Valentino M, Santiago-Schwarz F. Aberrant extracellular and dendritic cell (DC) surface expression of heat shock protein (hsp)70 in the rheumatoid joint: possible mechanisms of hsp/DC-mediated cross-priming. J Immunol 2003; 171(11): 5736-42.

[132] Kurzik-Dumke U, Schick C, Rzepka R, Melchers I. Overexpression of human homologs of the bacterial DnaJ chaperone in the synovial tissue of patients with rheumatoid arthritis. Arthritis Rheum 1999; 42(2): 210-20.

[133] Yokota SI, Hirata D, Minota S, *et al.* Autoantibodies against chaperonin CCT in human sera with rheumatic autoimmune diseases: comparison with antibodies against other Hsp60 family proteins. Cell Stress Chaperones 2000; 5(4): 337-46.

[134] Tsoulfa G, Rook GA, Bahr GM, *et al.* Elevated IgG antibody levels to the mycobacterial 65-kDa heat shock protein are characteristic of patients with rheumatoid arthritis. Scand J Immunol 1989; 30(5): 519-27.

[135] Bodman-Smith MD, Corrigall VM, Berglin E, *et al.* Antibody response to the human stress protein BiP in rheumatoid arthritis. Rheumatology (Oxford) 2004; 43(10): 1283-7.

[136] Goeb V, Thomas-L'Otellier M, Daveau R, *et al.* Candidate autoantigens identified by mass spectrometry in early rheumatoid arthritis are chaperones and citrullinated glycolytic enzymes. Arthritis Res Ther 2009; 11(2): R38.

[137] Auger I, Roudier J. Interaction between HSP73 and HLA-DRB1*0401: implications for the development of rheumatoid arthritis. Immunol Res 2005; 31(3): 261-6.

[138] Bodman-Smith MD, Corrigall VM, Kemeny DM, Panayi GS. BiP, a putative autoantigen in rheumatoid arthritis, stimulates IL-10-producing CD8-positive T cells from normal individuals. Rheumatology (Oxford) 2003; 42(5): 637-44.

[139] Barbera A, Lorenzo N, Garrido G, *et al.* APL-1, an altered peptide ligand derived from human heat-shock protein 60, selectively induces apoptosis in activated CD4+ CD25+ T cells from peripheral blood of rheumatoid arthritis patients. Int Immunopharmacol 2013; 17(4): 1075-83.

[140] Cuervo AM. Autophagy and aging: keeping that old broom working. Trends Genet 2008; 24(12): 604-12.

[141] Vicencio JM, Galluzzi L, Tajeddine N, *et al.* Senescence, apoptosis or autophagy? When a damaged cell must decide its path--a mini-review. Gerontology 2008; 54(2): 92-9.

[142] Xu K, Xu P, Yao JF, Zhang YG, Hou WK, Lu SM. Reduced apoptosis correlates with enhanced autophagy in synovial tissues of rheumatoid arthritis. Inflamm Res 2013; 62(2): 229-37.

[143] Shin YJ, Han SH, Kim DS, *et al.* Autophagy induction and CHOP under-expression promotes survival of fibroblasts from rheumatoid arthritis patients under endoplasmic reticulum stress. Arthritis Res Ther 2010; 12(1): R19.

[144] Kato M, Ospelt C, Gay RE, Gay S, Klein K. Dual role of autophagy in stress-induced cell death in rheumatoid arthritis synovial fibroblasts. Arthritis Rheumatol 2014; 66(1): 40-8.

[145] Connor AM, Mahomed N, Gandhi R, Keystone EC, Berger SA. TNFalpha modulates protein degradation pathways in rheumatoid arthritis synovial fibroblasts. Arthritis Res Ther 2012; 14(2): R62.

[146] Lin NY, Stefanica A, Distler JH. Autophagy: a key pathway of TNF-induced inflammatory bone loss. Autophagy 2013; 9(8): 1253-5.

[147] Izumi T, Fujii R, Izumi T, *et al.* Activation of synoviolin promoter in rheumatoid synovial cells by a novel transcription complex of interleukin enhancer binding factor 3 and GA binding protein alpha. Arthritis Rheum 2009; 60(1): 63-72.

[148] Kawaida R, Yamada R, Kobayashi K, *et al.* CUL1, a component of E3 ubiquitin ligase, alters lymphocyte signal transduction with possible effect on rheumatoid arthritis. Genes Immun 2005; 6(3): 194-202.

[149] Negi S, Kumar A, Thelma BK, Juyal RC. Association of Cullin1 haplotype variants with rheumatoid arthritis and response to methotrexate. Pharmacogenet Genomics 2011; 21(9): 590-3.

[150] Amano T, Yamasaki S, Yagishita N, *et al.* Synoviolin/Hrd1, an E3 ubiquitin ligase, as a novel pathogenic factor for arthropathy. Genes Dev 2003; 17(19): 2436-49.

[151] Toh ML, Marotte H, Blond JL, *et al.* Overexpression of synoviolin in peripheral blood and synoviocytes from rheumatoid arthritis patients and continued elevation in nonresponders to infliximab treatment. Arthritis Rheum 2006; 54(7): 2109-18.

[152] Yamasaki S, Yagishita N, Tsuchimochi K, Nishioka K, Nakajima T. Rheumatoid arthritis as a hyper-endoplasmic-reticulum-associated degradation disease. Arthritis Res Ther 2005; 7(5): 181-6.

[153] Takada K, Nasu H, Hibi N, *et al.* Serum concentrations of free ubiquitin and multiubiquitin chains. Clin Chem 1997; 43(7): 1188-95.

[154] Yang Z, Fujii H, Mohan SV, Goronzy JJ, Weyand CM. Phosphofructokinase deficiency impairs ATP generation, autophagy, and redox balance in rheumatoid arthritis T cells. J Exp Med 2013; 210(10): 2119-2134.

[155] Denko CW, Malemud CJ. Role of the growth hormone/insulin-like growth factor-1 paracrine axis in rheumatic diseases. Semin Arthritis Rheum 2005; 35(1): 24-34.

[156] Neidel J. Changes in systemic levels of insulin-like growth factors and their binding proteins in patients with rheumatoid arthritis. Clin Exp Rheumatol 2001; 19(1): 81-4.

[157] Sherlock M, Toogood AA. Aging and the growth hormone/insulin like growth factor-I axis. Pituitary 2007; 10(2): 189-203.

[158] Matsumoto T, Tsurumoto T. Inappropriate serum levels of IGF-I and IGFBP-3 in patients with rheumatoid arthritis. Rheumatology (Oxford) 2002; 41(3): 352-3.

[159] Ishigami S, Nakajima A, Tanno M, Matsuzaki T, Suzuki H, Yoshino S. Effects of mirthful laughter on growth hormone, IGF-1 and substance P in patients with rheumatoid arthritis. Clin Exp Rheumatol 2005; 23(5): 651-7.

[160] Blackman MR, Muniyappa R, Wilson M, *et al.* Diurnal secretion of growth hormone, cortisol, and dehydroepiandrosterone in pre- and perimenopausal women with active rheumatoid arthritis: a pilot case-control study. Arthritis Res Ther 2007; 9(4): R73.

[161] Dhaunsi GS, Uppal SS, Haider MZ. Insulin-like growth factor-1 gene polymorphism in rheumatoid arthritis patients. Scand J Rheumatol 2012; 41(6): 421-5.

[162] Lee HS, Woo SJ, Koh HW, *et al.* Regulation of apoptosis and inflammatory responses by IGFBP-3 in fibroblast-like synoviocytes and experimental animal models of rheumatoid arthritis. Arthritis Rheum 2013 Dec 10.

[163] Fraser DA, Thoen J, Reseland JE, Forre O, Kjeldsen-Kragh J. Decreased CD4+ lymphocyte activation and increased interleukin-4 production in peripheral blood of rheumatoid arthritis patients after acute starvation. Clin Rheumatol 1999; 18(5): 394-401.

[164] Toussirot E, Nguyen NU, Dumoulin G, Aubin F, Cedoz JP, Wendling D. Relationship between growth hormone-IGF-I-IGFBP-3 axis and serum leptin levels with bone mass and body composition in patients with rheumatoid arthritis. Rheumatology (Oxford) 2005; 44(1): 120-5.

[165] Suzuki S, Morimoto S, Fujishiro M, *et al.* Inhibition of the insulin-like growth factor system is a potential therapy for rheumatoid arthritis. Autoimmunity 2015; 48(4): 251-8.

[166] Lee SD, Chen LM, Kuo WW, *et al.* Serum insulin-like growth factor-axis and matrix metalloproteinases in patients with rheumatic arthritis or rheumatic heart disease. Clin Chim Acta 2006; 367(1-2): 62-8.

[167] Tavera C, Abribat T, Reboul P, *et al.* IGF and IGF-binding protein system in the synovial fluid of osteoarthritic and rheumatoid arthritic patients. Osteoarthritis Cartilage 1996; 4(4): 263-74.

[168] Neidel J, Blum WF, Schaeffer HJ, *et al.* Elevated levels of insulin-like growth factor (IGF) binding protein-3 in rheumatoid arthritis synovial fluid inhibit stimulation by IGF-I of articular chondrocyte proteoglycan synthesis. Rheumatol Int 1997; 17(1): 29-37.

[169] Matsumoto T, Yamashita S, Rosenfeld RG. Increased levels of IGF-I and IGFBP-3 in synovial fluids of patients with rheumatoid arthritis. Endocr J 1998; 45 (Suppl): S141-4.

[170] Templ E, Koeller M, Riedl M, Wagner O, Graninger W, Luger A. Anterior pituitary function in patients with newly diagnosed rheumatoid arthritis. Br J Rheumatol 1996; 35(4): 350-6.

[171] Volin MV, Huynh N, Klosowska K, Chong KK, Woods JM. Fractalkine is a novel chemoattractant for rheumatoid arthritis fibroblast-like synoviocyte signaling through MAP kinases and Akt. Arthritis Rheum 2007; 56(8): 2512-22.

[172] Garcia S, Liz M, Gomez-Reino JJ, Conde C. Akt activity protects rheumatoid synovial fibroblasts from Fas-induced apoptosis by inhibition of Bid cleavage. Arthritis Res Ther 2010; 12(1): R33.

[173] Grabiec AM, Angiolilli C, Hartkamp LM, van Baarsen LG, Tak PP, Reedquist KA. JNK-dependent downregulation of FoxO1 is required to promote the survival of fibroblast-like synoviocytes in rheumatoid arthritis. Ann Rheum Dis 2014 May 8.[Epub ahead of Print].

[174] Ludikhuize J, de Launay D, Groot D, *et al.* Inhibition of forkhead box class O family member transcription factors in rheumatoid synovial tissue. Arthritis Rheum 2007; 56(7): 2180-2191.

[175] Malemud CJ. Intracellular signaling pathways in rheumatoid arthritis. J Clin Cell Immunol 2013; 4: 160.

[176] Saxena A, Raychaudhuri SK, Raychaudhuri SP. Interleukin-17-induced proliferation of fibroblast-like synovial cells is mTOR dependent. Arthritis Rheum 2011; 63(5): 1465-6.

[177] Da Sylva TR, Connor A, Mburu Y, Keystone E, Wu GE. Somatic mutations in the mitochondria of rheumatoid arthritis synoviocytes. Arthritis Res Ther 2005; 7(4): R844-51.

[178] Harty LC, Biniecka M, O'Sullivan J, *et al.* Mitochondrial mutagenesis correlates with the local inflammatory environment in arthritis. Ann Rheum Dis 2012; 71(4): 582-8.

[179] Valcarcel-Ares MN, Riveiro-Naveira RR, Vaamonde-Garcia C, *et al.* Mitochondrial dysfunction promotes and aggravates the inflammatory response in normal human synoviocytes. Rheumatology (Oxford) 2014; 53(7): 1332-43.

[180] Hajizadeh S, DeGroot J, TeKoppele JM, Tarkowski A, Collins LV. Extracellular mitochondrial DNA and oxidatively damaged DNA in synovial fluid of patients with rheumatoid arthritis. Arthritis Res Ther 2003; 5(5): R234-40.

[181] Collins LV, Hajizadeh S, Holme E, Jonsson IM, Tarkowski A. Endogenously oxidized mitochondrial DNA induces *in vivo* and in vitro inflammatory responses. J Leukoc Biol 2004; 75(6): 995-1000.

[182] van Deursen JM. The role of senescent cells in ageing. Nature 2014; 509(7501): 439-46.

[183] Alexander E, Hildebrand DG, Kriebs A, *et al.* IkappaBzeta is a regulator of the senescence-associated secretory phenotype in DNA damage- and oncogene-induced senescence. J Cell Sci 2013; 126(Pt 16): 3738-45.

[184] Tak PP, Smeets TJ, Boyle DL, *et al.* P53 Overexpression in synovial tissue from patients with early and longstanding rheumatoid arthritis compared with patients with reactive arthritis and osteoarthritis. Arthritis Rheum 1999; 42(5): 948-53.

[185] Yamanishi Y, Boyle DL, Rosengren S, Green DR, Zvaifler NJ, Firestein GS. Regional analysis of p53 mutations in rheumatoid arthritis synovium. Proc Natl Acad Sci USA 2002; 99(15): 10025-30.

[186] Lee CS, Portek I, Edmonds J, Kirkham B. Synovial membrane p53 protein immunoreactivity in rheumatoid arthritis patients. Ann Rheum Dis 2000; 59(2): 143-5.

[187] Murakami Y, Mizoguchi F, Saito T, Miyasaka N, Kohsaka H. p16(INK4a) exerts an anti-inflammatory effect through accelerated IRAK1 degradation in macrophages. J Immunol 2012; 189(10): 5066-72.

[188] Taniguchi K, Kohsaka H, Inoue N, *et al.* Induction of the p16INK4a senescence gene as a new therapeutic strategy for the treatment of rheumatoid arthritis. Nat Med 1999; 5(7): 760-7.

[189] Carson DA, Haneji N. Fighting arthritis with a senescence gene. Nat Med 1999; 5(7): 731-2.

[190] Lee BY, Han JA, Im JS, *et al.* Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. Aging Cell 2006; 5(2): 187-95.

[191] Harvey AR, Clarke BJ, Chui DH, Kean WF, Buchanan WW. Anemia associated with rheumatoid disease. Inverse correlation between erythropoiesis and both IgM and rheumatoid factor levels. Arthritis Rheum 1983; 26(1): 28-34.

[192] Reid CD, Prouse PJ, Baptista LC, Gumpel JM, Chanarin I. The mechanism of the anaemia in rheumatoid arthritis: effects of bone marrow adherent cells and of serum on in-vitro erythropoiesis. Br J Haematol 1984; 58(4): 607-15.

[193] Sugimoto M, Wakabayashi Y, Hirose S, Takaku F. Immunological aspects of the anemia of rheumatoid arthritis. Am J Hematol 1987; 25(1): 1-11.

[194] Papadaki HA, Kritikos HD, Gemetzi C, *et al.* Bone marrow progenitor cell reserve and function and stromal cell function are defective in rheumatoid arthritis: evidence for a tumor necrosis factor alpha-mediated effect. Blood 2002; 99(5): 1610-19.

[195] Papadaki HA, Kritikos HD, Valatas V, Boumpas DT, Eliopoulos GD. Anemia of chronic disease in rheumatoid arthritis is associated with increased apoptosis of bone marrow erythroid cells: improvement following anti-tumor necrosis factor-alpha antibody therapy. Blood 2002; 100(2): 474-82.

[196] Porta C, Caporali R, Epis O, *et al.* Impaired bone marrow hematopoietic progenitor cell function in rheumatoid arthritis patients candidated to autologous hematopoietic stem cell transplantation. Bone Marrow Transplant 2004; 33(7): 721-8.

[197] Colmegna I, Diaz-Borjon A, Fujii H, Schaefer L, Goronzy JJ, Weyand CM. Defective proliferative capacity and accelerated telomeric loss of hematopoietic progenitor cells in rheumatoid arthritis. Arthritis Rheum 2008; 58(4): 990-1000.

[198] Colmegna I, Pryshchep S, Oishi H, Goronzy JJ, Weyand CM. Dampened ERK signaling in hematopoietic progenitor cells in rheumatoid arthritis. Clin Immunol 2012; 143(1): 73-82.

[199] McCubrey JA, Steelman LS, Chappell WH, *et al.* Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta 2007; 1773(8): 1263-84.

[200] Fujii H, Shao L, Colmegna I, Goronzy JJ, Weyand CM. Telomerase insufficiency in rheumatoid arthritis. Proc Natl Acad Sci USA 2009; 106(11): 4360-65.

[201] Masi AT, Rehman AA, Elmore KB, Aldag JC. Serum acute phase protein and inflammatory cytokine network correlations: comparison of a pre-rheumatoid arthritis and non-rheumatoid arthritis community cohort. J Innate Immun 2013; 5(2): 100-13.

[202] Park J, Choi HM, Yang HI, Yoo MC, Kim KS. Increased expression of IL-1 receptors in response to IL-1beta may produce more IL-6, IL-8, VEGF, and PGE(2) in senescent synovial cells induced in vitro than in presenescent cells. Rheumatol Int 2012; 32(7): 2005-10.

[203] Rodier F, Coppe JP, Patil CK, *et al.* Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol 2009; 11(8): 973-9.

[204] Correia-Melo C, Hewitt G, Passos JF. Telomeres, oxidative stress and inflammatory factors: partners in cellular senescence? Longev Healthspan 2014; 3(1): 1-2395-3-1.

[205] Fumagalli M, d'Adda di Fagagna F. SASPense and DDRama in cancer and ageing. Nat Cell Biol 2009; 11(8): 921-3.

[206] Kageyama Y, Takahashi M, Nagafusa T, Torikai E, Nagano A. Etanercept reduces the oxidative stress marker levels in patients with rheumatoid arthritis. Rheumatol Int 2008; 28(3): 245-51.

[207] Kageyama Y, Takahashi M, Ichikawa T, Torikai E, Nagano A. Reduction of oxidative stress marker levels by anti-TNF-alpha antibody, infliximab, in patients with rheumatoid arthritis. Clin Exp Rheumatol 2008; 26(1): 73-80.

[208] Toussirot E, Wendling D, Herbein G, on behalf of CIC-1431. Biological treatments given in patients with rheumatoid arthritis or ankylosing spondylitis modify HAT/HDAC (histone acetyltransferase/histone deacetylase) balance. Joint Bone Spine 2014; 81(6): 544-5.

[209] Wagner U, Schatz A, Baerwald C, Rossol M. Brief report: deficient thymic output in rheumatoid arthritis despite abundance of prethymic progenitors. Arthritis Rheum 2013; 65(10): 2567-72.

[210] Bryl E, Vallejo AN, Matteson EL, Witkowski JM, Weyand CM, Goronzy JJ. Modulation of CD28 expression with anti-tumor ne-

crosis factor alpha therapy in rheumatoid arthritis. Arthritis Rheum 2005; 52(10): 2996-3003.

[211] Bryl E, Vallejo AN, Weyand CM, Goronzy JJ. Down-regulation of CD28 expression by TNF-alpha. J Immunol 2001; 167(6): 3231-38.

[212] Gomez-Garcia L, Ramirez-Assad C, Vargas A, *et al.* Reduced numbers of circulating CD28-negative CD4+ cells in patients with rheumatoid arthritis chronically treated with abatacept. Int J Rheum Dis 2013; 16(4): 469-71.

[213] Scarsi M, Ziglioli T, Airo P. Decreased circulating CD28-negative T cells in patients with rheumatoid arthritis treated with abatacept are correlated with clinical response. J Rheumatol 2010; 37(5): 911-16.

[214] Scarsi M, Ziglioli T, Airo' P. Baseline numbers of circulating CD28-negative T cells may predict clinical response to abatacept in patients with rheumatoid arthritis. J Rheumatol 2011; 38(10): 2105-11.

[215] Parish ST, Wu JE, Effros RB. Modulation of T lymphocyte replicative senescence via TNF-{alpha} inhibition: role of caspase-3. J Immunol 2009; 182(7): 4237-43.

[216] Laberge RM, Zhou L, Sarantos MR, *et al.* Glucocorticoids suppress selected components of the senescence-associated secretory phenotype. Aging Cell 2012; 11(4): 569-78.

[217] Kageyama Y, Takahashi M, Nagafusa T, Torikai E, Nagano A. Methotrexate reduces the levels of pentosidine and 8-hydroxydeoxy guanosine in patients with rheumatoid arthritis. Mod Rheumatol 2007; 17(5): 398-402.

Received: January 06, 2015 **Revised: May 18, 2015** **Accepted: June 12, 2015**

[218] Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. Lancet 2009; 373(9664): 659-72.

[219] Kokkonen H, Soderstrom I, Rocklov J, Hallmans G, Lejon K, Rantapaa Dahlqvist S. Up-regulation of cytokines and chemokines predates the onset of rheumatoid arthritis. Arthritis Rheum 2010; 62(2): 383-91.

[220] Sokolove J, Johnson DS, Lahey LJ, *et al.* Rheumatoid factor as a potentiator of anti-citrullinated protein antibody-mediated inflammation in rheumatoid arthritis. Arthritis Rheumatol 2014; 66(4): 813-21.

[221] Clavel C, Nogueira L, Laurent L, *et al.* Induction of macrophage secretion of tumor necrosis factor alpha through Fcgamma receptor IIa engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen. Arthritis Rheum 2008; 58(3): 678-88.

[222] Laurent L, Clavel C, Lemaire O, *et al.* Fcgamma receptor profile of monocytes and macrophages from rheumatoid arthritis patients and their response to immune complexes formed with autoantibodies to citrullinated proteins. Ann Rheum Dis 2011; 70(6): 1052-9.

[223] van de Stadt LA, Witte BI, Bos WH, van Schaardenburg D. A prediction rule for the development of arthritis in seropositive arthralgia patients. Ann Rheum Dis 2012 Nov 28.

[224] van der Helm-van Mil AH. Risk estimation in rheumatoid arthritis: from bench to bedside. Nat Rev Rheumatol 2014; 10(3): 171-80.