Experimental Gerontology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Review

Senescent B cells in aging and age-related diseases: Their role in the regulation of antibody responses

Daniela Frasca

Department of Microbiology and Immunology, Room #3146A, University of Miami Miller School of Medicine, P.O. Box 016960 (R-138), Miami, FL 33101, USA.

ARTICLE INFO

Article history:
Received 1 June 2017
Received in revised form 29 June 2017
Accepted 1 July 2017
Available online xxxx

Section Editor: Chennai Guest Editor

Keywords:
Antibody responses
B cells
Inflammation
Cell senescence

ABSTRACT

Immune cells with a senescence-associated secretory phenotype increase in the blood of elderly individuals or individuals with age-associated diseases or with infections. Although senescent immune cells do not proliferate, they are transcriptionally and metabolically active and affect the microenvironment through the secretion of pro-inflammatory mediators. An age-driven increase in senescent B, T and NK cells has been reported and the function of these cells has been characterized. Results published by different groups have demonstrated that cell senescence induces the accumulation of terminally-differentiated cells characterized by the arrest of cell proliferation but with an active secretory profile which regulates their function through the activation of pathways integrating senescence and energy-sensing signals. This review will focus on senescent B cells, their increase in aging, age-associated conditions and infections. Similarities with other senescent immune cells will be presented and discussed.

Contents

1. Introduction

Aging is characterized by increased low-grade chronic inflammation, called "inflammaging" (Franceschi et al., 2000), which represents a significant risk factor for morbidity and mortality of elderly individuals as it is implicated in the pathogenesis of several disabling diseases of the elderly, including Type-2 Diabetes, osteoporosis, Alzheimer's disease, Rheumatoid Arthritis, atherosclerosis and coronary heart disease (Alexopoulos et al., 2014; Holmes et al., 2009; Isaacs, 2009; Libby, 2012; Lindholm et al., 2008; Mundy, 2007; Sarzi-Puttini et al., 2005). Circulating inflammatory mediators such as cytokines and acute phase proteins, are markers of inflammaging. Among these, elevated serum levels of IL-6 and C-Reactive Protein, have been shown to predict 3-year mortality in the elderly by the Invecchiare in Chianti study (Alley et al., 2007).

The ways in which inflammaging contributes to adverse health outcomes is not completely understood, and therefore the identification of pathways controlling inflammaging across multiple systems is important, in order to design protocols of intervention to reduce inflammaging and potentially improve the health of elderly individuals.

Several factors contribute to inflammaging, including polymorphisms in the promoter regions of pro-inflammatory genes, chronic stimulation of immune cells with viruses such as cytomegalovirus (CMV), obesity, changes in the gut microbiome, increased permeability from the intestine [reviewed in (Frasca and Blomberg, 2016)]. Cellular senescence has also been proposed to be a significant contributor to inflammaging, due to the acquisition of the senescence-associated secretory phenotype (SASP) by fibroblasts (Freund et al., 2010), endothelial

E-mail address: dfrasca@med.miami.edu.

http://dx.doi.org/10.1016/j.exger.2017.07.002
0531-5565/

cells (Olivieri et al., 2013) and immune cells (Sikora et al., 2011). A large-scale characterization of the SASP has been performed in fibroblasts and endothelial cells using antibody arrays to quantitatively measure pro-inflammatory mediators, such as cytokines, chemokines, micro-RNAs, growth factors and proteases (Campisi, 2011). Work under way is aiming at thoroughly characterize the senescent secretome of immune cells.

Senescent cells are characterized by the arrest of cell proliferation and have short telomeres, but they are transcriptionally and metabolically active. This high activity of senescent cells derives from the SASP, which leads to the secretion of multiple factors with potent biological activities on surrounding cells and tissues.

The term "senescence" has recently generated controversy within the aging field, especially because the arrest of cell proliferation has been observed in highly differentiated immune cells which can secrete multiple factors regulating their function. In the case of B cells, these highly differentiated cells represent the terminally-differentiated subset, as they derive from IgM or switched memory B cells (Bagnara et al., 2015). This process of differentiation seems to occur through the activation of non canonical pathways integrating senescence (Frasca et al., 2017a) and energy-sensing signals (Torigoe et al., 2017), similar to what has been shown for T (Henson et al., 2014; Henson et al., 2015; Lanna et al., 2014) and NK (Muller-Durovic et al., 2016) cells.

Changes in glucose levels in the extracellular milieu occur with age (Spazzafumo et al., 2013), and may be responsible for decreased function, as B cells utilize glucose for proliferation and differentiation (Caro-Maldonado et al., 2014), as it has also been shown for T cells (Heikamp and Powell, 2012; Verbist et al., 2012; Xu et al., 2012) and macrophages (Recalcati et al., 2012). Briefly, T cell activation leads to metabolic reprogramming characterized by a rapid increase in the expression of glucose transporters to support glycolysis over oxidative metabolism and pathways of this metabolic reprogramming have been characterized (Frauwirth et al., 2002; Rathmell, 2012). Similar to T cells, B cells also increase the expression of glucose transporters and mitochondrial mass upon mitogen or antigen stimulation, and deletion of glucose transporters reduces B cell numbers and antibody production (Caro-Maldonado et al., 2014). Experiments in our laboratory are currently evaluating the energy metabolism of the different B cell subsets, by performing gene expression analysis of pathways related to the glucose metabolism, measuring glucose uptake upon cell stimulation, and determining mitochondrial morphology, abundance and function.

2. Senescent B cell subsets are increased in the blood of healthy elderly individuals

Inflammaging is associated with changes in the distribution of B cell subsets in the peripheral blood. Four major peripheral B cell subsets can be measured by flow cytometry in the human blood: naive (IgD+/CD27−), IgM memory (IgD+/CD27+), switched memory (IgD−/CD27+), late memory (LM, IgD−/CD27−). We have shown that LM B cells are increased in percentages (and numbers) in the blood of healthy elderly *versus* young individuals (Frasca et al., 2017a; Frasca et al., 2017b; Frasca et al., 2016), similar to what has also been reported by other groups (Martorana et al., 2014; Rinaldi et al., 2017). Phenotypic and functional characteristics of LM B cells are summarized in Table 1.

The frequency of LM B cells in blood has been found to be negatively associated with a protective response against the influenza vaccine, measured by hemagglutination inhibition assay at t28 (1 month after influenza vaccination) (Frasca et al., 2017a), or by the frequency of plasmablasts in blood at t7 (1 week after influenza vaccination) (Rinaldi et al., 2017). Both measures represent good correlates of vaccine protection. This negative association was expected, based on the fact that this B cell subset is highly inflammatory and has been reported to show characteristics of cell senescence, such as poor ability to proliferate *in vitro* in response to mitogenic stimulation and reduced telomerase activity (Colonna-Romano et al., 2009; Martorana et al., 2014).

Table 1
Phenotypic and functional characteristics of LM B cells from healthy individuals.

Measure	References
Membrane markers of immune activation	
$CD95^{high}$	Adlowitz et al. (2015), Frasca (2017b)
$CD21^{low}$	Adlowitz et al. (2015), Claes et al. (2016), Frasca (2017b)
CD11c	Claes et al. (2016), Frasca (2017b)
Chemokine receptors	
CXCR3	Bulati et al. (2014)
Inflammatory cytokines/chemokines	
TNF-α/IL-6/IL-8	Frasca (2017a)
Inflammatory micro-RNAs (miRs)	
miR-155, miR-16, miR-93	Frasca (2017a)
Cell cycle regulators	
$p16^{INK4}$	Frasca (2017a)
Telomere length	
Short	Colonna-Romano et al. (2009), Martorana et al. (2014)
Cell proliferation	
Reduced	Colonna-Romano et al. (2009)
Spontaneous AMPK/p38MAPK/NF-kB activation	
High	Frasca (2017a)
T-bet expression	
High	Chang et al. (2016), Frasca (2017b)
Class switch and antibody secretion	Frasca (2017a), Frasca (manuscript in preparation)
Reduced	

Results indicate changes in expression/function as compared to the other B cell subsets (naïve, IgM memory, switched memory) from both young and elderly individuals.

Although LM B cells do not proliferate *in vitro* in response to mitogenic stimulation, they are transcriptionally active. In our recently published work (Frasca et al., 2017a), we have evaluated the functional quality of the B cell pool, as this influences the individual's response. We have shown that unstimulated memory but not naïve B cells from both young and elderly individuals, evaluated at t0 (before vaccination), express RNA for multiple SASP markers, such as the pro-inflammatory cytokines TNF-α/IL-6/IL-8 and for the pro-inflammatory micro-RNAs (miRs)-155/16/93. Levels are higher in B cells from elderly *versus* young individuals. Among memory B cell subsets, the LM subset expresses the highest level of SASP markers.

Unstimulated memory but not naïve B cells from both young and elderly individuals also express RNA for $p16^{INK4}$ with the LM subset showing the highest levels of this SASP marker. The fact that switched memory and IgM memory B cells from elderly individuals show higher levels of expression of SASP markers as compared to younger individuals may help to explain their decreased function in the elderly. Through secretion of these pro-inflammatory mediators, LM B cells affect the microenvironment and in turn sustain and propagate the inflammatory response and negatively regulate the function of other immune cells. We have indeed previously shown that the levels of endogenous TNF-α in B cells negatively impact their ability to proliferate, differentiate and generate optimal antibody responses (Frasca et al., 2014). These results demonstrate that basal (pre-stimulation) levels of TNF-α in B cells negatively impact the ability of the same B cells to generate optimal function. Moreover, pre-incubation of B cells with an anti-TNF-α antibody, before *in vitro* stimulation, significantly increase B cell function, indicating that it is possible to improve B cell function and antibody production by counteracting intrinsic levels of TNF-α (Frasca et al., 2014). Recent evidence from our laboratory has shown that if LM cells are sorted out from the total B cell pool, class switch increases, and more in individuals with high endogenous levels of TNF-α (manuscript in preparation).

LM B cells are also characterized by $CD95^{high}$, $CD21^{low}$, T-bet and CD11c expression as compared to the other B cell subsets (Frasca et al., 2017b). Up-regulation of CD95 (Fas ligand) (Jacobi et al., 2008)

Please cite this article as: Frasca, D., Senescent B cells in aging and age-related diseases: Their role in the regulation of antibody responses, Exp. Gerontol. (2017), http://dx.doi.org/10.1016/j.exger.2017.07.002