Experimental Gerontology 87 (2017) 113–120

Contents lists available at ScienceDirect

Experimental Gerontology

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

Human peripheral late/exhausted memory B cells express a senescent-associated secretory phenotype and preferentially utilize metabolic signaling pathways

Daniela Frasca *, Alain Diaz, Maria Romero, Bonnie B. Blomberg

Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL 33101, USA

ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form 7 November 2016
Accepted 2 December 2016
Available online 06 December 2016

Keywords:
Aging
B cells
Inflammation
Vaccine responses
SASP

ABSTRACT

The percentage of late/exhausted memory (LM) B cells increases with age and we show here that this is associated with a lower influenza vaccine response. To identify novel contributors to the phenotypic and functional changes observed in aged B cells, we sorted the major peripheral B cell subsets [naïve, IgM memory, switched memory (swIg) and late/exhausted memory (LM)] and determined their percentages in the peripheral blood as well as their level of immune activation by measuring basal levels of expression of multiple senescence-associated secretory phenotype (SASP) markers, such as pro-inflammatory cytokines (TNF-α/IL-6/IL-8), inflammatory micro-RNAs (miRs, miR-155/16/93), cell cycle regulators ($p16^{INK4}$). We found that only memory B cells express SASP markers, and especially the LM B cell subset, which is also showing spontaneous activation of AMP-activated protein kinase (AMPK), the energy sensing enzyme which is ubiquitously expressed in mammalian cells. LM B cells, but not IgM memory B cells, activate a p38MAPK signaling pathway, downstream of AMPK, leading to the expression of SASP mediators, while class switch recombination is downregulated. These data show that some B cell subsets are more inflammatory than others, that they are pre-activated and that this signaling through metabolic pathways is associated with a senescence phenotype, demonstrating for the first time in human B lymphocytes the link between aging, cellular senescence, SASP and metabolism.

1. Introduction

Aging represents a complex remodeling in which innate and adaptive immune responses deteriorate, leading to greater susceptibility to infectious diseases and reduced responses to vaccines (Boraschi et al., 2010). Aging is characterized by increased low-grade chronic inflammation, called "inflammaging", measured by circulating levels of pro-inflammatory mediators (TNF-α, IL-6, CRP), as well as by latent infections with viruses such as cytomegalovirus (CMV). Inflammaging is 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, such as type-2 diabetes mellitus (Lindholm et al., 2008), osteoporosis (Mundy, 2007), Alzheimer's disease (Holmes et al., 2009), rheumatoid arthritis (Isaacs, 2009), and coronary heart disease (Sarzi-Puttini et al., 2005).

Cellular senescence could be a significant contributor to inflammaging, due to the acquisition of the senescence-associated secretory phenotype (SASP) by cells of the immune system (Sikora et al., 2011), fibroblasts (Coppe et al., 2008; Freund et al., 2010) and endothelial cells (Olivieri et al., 2013), characterized by increased secretion of pro-inflammatory mediators such as cytokines, chemokines, growth factors and proteases (Campisi, 2011). The age-dependent accumulation of senescent cells represents a favorable environment for the development of inflammatory-based age-related diseases (cancer, cardiovascular and neurodegenerative disease). At the same time, however, senescence may suppress the development of cancer by stopping the growth of premalignant cells (Campisi, 2003).

Aging and inflammation decrease antibody responses, due to effects on T cells (Goronzy et al., 2001; Gupta et al., 2004; Pawelec et al., 2002), B cells (Frasca et al., 2014; Frasca et al., 2015b; Gibson et al., 2009) and antigen-presenting cells (Panda et al., 2010; Sridharan et al., 2011). We have analyzed the influenza vaccine response in healthy individuals of different ages as well as the composition and quality of their peripheral B cell pool, as we have shown that both influence the individual's response (Frasca et al., 2014; Frasca et al., 2015b; Frasca et al., 2016).

In this report, we show previously undescribed novel inflammatory markers seen in aging B cells and which is the major inflammatory B cell subset. We also show that markers of the SASP phenotype previously seen predominantly in non immune cells are also present in B lymphocytes. We have sorted the 4 main peripheral B cell subsets [naïve, IgM memory, switched memory (swIg) and late/exhausted memory (LM)] and determined their percentages in the peripheral blood as well as their level of immune activation by measuring basal levels of expression of multiple SASP markers, such as pro-inflammatory cytokines (TNF-α/

* Corresponding author at: Department of Microbiology and Immunology, University of Miami Miller School of Medicine, P.O. Box 016960 (R-138), Miami, FL 33101, USA.
E-mail address: dfrasca@med.miami.edu (D. Frasca).

http://dx.doi.org/10.1016/j.exger.2016.12.001
0531-5565/

IL-6/IL-8), cell cycle regulators ($p16^{INK4}$), inflammatory micro-RNAs (miRs, miR-155/16/93). These miRs were selected because we have shown that their levels negatively correlate with optimal B cell responses (Frasca et al., 2015a). We found that only memory B cells express SASP markers, and especially the LM B cell subset, which is also showing spontaneous activation of AMP-activated protein kinase (AMPK), the energy sensing enzyme, a key metabolic regulator ubiquitously expressed in mammalian cells (Ruderman and Prentki, 2004). The LM B cells, previously identified in healthy individuals (Fecteau et al., 2006) and in patients with SLE (Anolik et al., 2004; Wei et al., 2007), have also been called double negative (DN). Similar to terminally differentiated CD4+ T cells (Akbar et al., 2016; Henson et al., 2014; Henson et al., 2015; Lanna et al., 2014) and NK cells (Muller-Durovic et al., 2016), LM B cells, but not IgM memory B cells, activate a p38MAPK signaling pathway, downstream of AMPK, leading to the secretion of SASP mediators, while class switch recombination and antibody production are downregulated. These data show for the first time that some B cell subsets are more inflammatory than others and that signaling through metabolic pathways is associated with a senescence phenotype.

2. Materials and methods

2.1. Subjects

Experiments were conducted using blood isolated from young (25–55 years) and elderly (≥65 years) healthy individuals, after appropriate signed informed consent and were approved with IRB protocol #20070481. The individuals participating in the study were screened for diseases known to alter the immune response or for consumption of medications that could alter the immune response. In particular, the following categories were excluded: established diagnosis of diabetes; one or more of the following co-morbid conditions including malignancy, Congestive Heart Failure, Cardiovascular Disease, Chronic Renal Failure, no renal or hepatic diseases, autoimmune diseases, infectious disease, recent (<3 months) trauma or surgery, pregnancy, or documented current substance and/or alcohol abuse. Demographic and serological characteristics of the participants are in Table 1.

Table 1
Demographic characteristics, inflammatory profile and vaccine responses of the participants.

	Young[a]	Elderly[a]
Participants (n)	6	6
Age (mean years ± SE)	36 ± 5	68 ± 51**
Gender (M/F)	3/3	4/2
Race (W/B)	4/2	4/2
Ethnic categories (Hispanic/non Hispanic)[b]	3/3	2/4
Influenza vaccine response		
Fold-increase in titers after vaccination[c]	7.7 ± 2.8	1.5 ± 0.9
Reciprocal of the titers at t7[c]	227 ± 44	57 ± 11**
TNF-α (pg/ml)	7 ± 1	15 ± 3*
IL-6 (pg/ml)	61 ± 10	133 ± 28**
CRP (pg/ml)	485 ± 75	11,124 ± 25*

Young: 25–55 years, elderly: ≥65 years.
[a] Prevalence of CMV-seropositivity was higher in elderly as compared to young individuals ($p < 0.05$). All subjects were CMV IgM-negative, which indicates chronic CMV infection and no viral reactivation.
[b] All races. Hispanic are individuals from Mexico, Puerto Rico, Cuba, Central/South America and from other countries with Spanish culture or origin. All plasma inflammatory cytokines were measured by ELISA and results are means ± SE. Normal plasma levels of TNF-α, IL-6, CRP are, respectively, 3–10 pg/ml, 30–60 pg/ml, ≤800 pg/ml.
[c] Responses are evaluated by HAI at t7 after vaccination.
** $p < 0.01$.
* $p < 0.05$.

2.2. Influenza vaccination

The study was conducted during the 2011–2012 seasonal influenza vaccination. Blood samples were collected immediately before (t0), 1 week (t7) and 4–6 (t28) weeks after vaccination. The 2011–2012 vaccination season was characterized by a vaccine containing the pandemic (p)2009 H1N1 strain for the third consecutive year. Viral strains were: A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2), and B/Brisbane/60/2008. All of our subjects were immunized in the previous pandemic 2009 and seasonal 2010–2011 seasons, and therefore seroprotected at t0. The peak of the antibody response was at t7, earlier than what we (Frasca et al., 2010; Frasca et al., 2012) and others (Verma et al., 2012) previously found, due to repeated vaccine immunizations. In most cases, peak titers were maintained through t28.

2.3. Hemagglutination inhibition (HAI) assay

The *in vivo* response was measured by HAI assay and results expressed as the reciprocal of specific titers after vaccination, as we have previously described (Frasca et al., 2010; Frasca et al., 2012). The HAI assay is based on the ability of certain viruses or viral components to hemagglutinate the red blood cells of specific animal species (Ito et al., 1997). Antibodies specific to influenza inhibit this agglutination. Paired pre- and post-immunization serum samples from the same individual were tested simultaneously. Briefly, sera were pretreated with receptor destroying enzyme (Denke Seiken Co Ltd) for 20 h at 37 °C; in order to inactivate this enzyme, sera were then heated at 56 °C for 60 min. Two-fold serial dilutions were done; 25 μl of diluted sera were incubated with an equal volume of 4 HA units of the vaccine, for 1 h at room temperature and then 50 μl of a 1.25% suspension of chicken red blood cells were added. After two hrs of incubation at room temperature titers were determined. Sera inhibiting titers of 1/40 or greater are defined as the positive measure of seroprotection against infection, whereas a four-fold rise in the reciprocal of the titer from t0 to t28 indicates a positive response to the vaccine and indicates seroconversion (Frasca et al., 2010; Frasca et al., 2012; Ito et al., 1997). We have previously shown that serum antibody responses measured by HAI were correlated with those measured by ELISA (Frasca et al., 2012).

2.4. Enzyme-linked immunosorbent assay (ELISA)

Plasma TNF-α, IL-6, CRP were measured by the following ELISA kits: Life Technologies KHC3013, KHC0062, KHA0032, respectively, following manufacturer's instructions.

2.5. Flow cytometry

One hundred μl of blood were stained for 20 min at room temperature with the following antibodies: anti-CD19 (BD 555415), anti-CD27 (BD 555441), anti-IgD (BD 555778) to measure naive (IgD+CD27−), IgM memory (IgD+CD27+), switched memory (swIg, IgD−CD27+), late/exhausted memory (LM, IgD−CD27−) B cells. After staining, red blood cells were lyzed using the RBC Lysing Solution (BD 555899), according to the manufacturer's instructions. Up to 10^5 events in the lymphocyte gate were acquired on an LSR-Fortessa (BD) and analyzed using FACS Diva (BD) software. Single color controls were included in every experiment for compensation. A representative dot plot to evaluate the different B cell subsets is shown in Fig. 1F.

2.6. B cell sorting

PBMC were collected by density gradient centrifugation using Vacutainer CPT tubes (BD 362761). PBMC were stained with anti-CD19, anti-CD27, anti-IgD antibodies and sorted using a FACS Aria (BD). Cell preparations were typically >98% pure.