Cellular Immunology 256 (2009) 1–5

Contents lists available at ScienceDirect

Cellular Immunology

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

Rapid Communication

Specific immune responses but not basal functions of B and T cells are impaired in aged mice

E. Speziali, A.F. Santiago, R.M. Fernandes, N.M. Vaz, J.S. Menezes, A.M.C. Faria *

Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901 Belo Horizonte, MG, Brazil

ARTICLE INFO

Article history:
Received 17 November 2008
Accepted 21 January 2009
Available online 23 February 2009

Keywords:
Aging
Immunoglobulin
T cell proliferation
Immunoglobulin-producing cells

ABSTRACT

Senescence is characterized by several alterations in the immune system. Such modifications can be found in lymphoid organs as well as in the cellular components of the immune system. Several reports have suggested that immune dysfunction can affect both T and B cells, but T cells have been shown to be more susceptible to the effects of aging. B cell function may also be altered with reduction in germinal center formation, antibody response, and affinity maturation of antibodies. Herein we showed that although antigen-specific antibody response to a soluble antigen declines in 18-month old mice, total levels of serum antibodies as well as frequencies of spleen and bone marrow antibody-producing cells are increased in aged mice. In addition, proliferative response of non-stimulated spleen T cells from aged mice were augmented and insensitive to increasing doses of concanavalin A stimulation as compared to young mice that showed a typical dose-dependent response to mitogen stimulation *in vitro*. These data suggest that the higher activation mode of B and T cells in senescent mice is a result of an increased frequency of cells committed to previous antigenic experiences and with poor ability to respond to novel antigenic challenges.

1. Introduction

Immune-senescence is a well-described phenomenon in humans and in many other animal species. Aging-associated immunological alterations can be found in lymphoid organs as well as in cellular components of immune system [1]. These changes have been extensively studied in systemic compartments and, although immunological dysfunction can affect both T and B cells, T cells are more susceptible to the effects of aging [2,3]. This higher susceptibility of T cells to aging is believed to result from the progressive loss of thymus function during aging with the consequent decrease in the output of naive T cells into the peripheral T cell pool [2]. Since the total number of peripheral T cells and TCD4/TCD8 rate are preserved, aging is followed by a progressive accumulation of T cells with a memory phenotype and a drastic reduction in the global T cell repertoire. All these changes result ultimately in a considerable decrease in cellular immune responses during aging [2,4]. In addition, naïve CD4 T cells in aged animals show decreased proliferation and IL-2 production as well as poor ability to differentiate in Th1 or Th2 subsets [5,6]. B cell function is altered with reduction in germinal center formation and antibody response. Antibody affinity maturation is also decreased in aged animal [7]. In spite of these aging associated modifications, it is still unclear whether senescence is followed by changes in serum immunoglobulin levels. [8–11]. In this study, we investigated, in aged mice, global B and T cell functions as compared to specific humoral and cellular immune responses that would require these two lymphocyte populations. We found that although some antigen-specific antibody responses declined, total antibody production, frequency of antibody-producing cells and proliferative response of spleen T cells were enhanced in aged mice.

2. Materials and methods

2.1. Animals

Female BALB/c and B6D2F1 mice (8–100 weeks old) were obtained from our animal facility (CEBIO, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, Brazil) and maintained in our conventional experimental animal facility throughout the experiments. All animal procedures were approved by the local ethical committee for animal research (CETEA-UFMG, Brazil).

2.2. Antigen and adjuvant

Crystallized hen's egg albumin (OVA, grade III or V, Sigma, St. Louis, MO) was used as antigen. Aluminum hydroxide [$Al(OH)_3$] was used as adjuvant.

* Corresponding author. Fax: +55 31 3409 2640.
E-mail address: afaria@icb.ufmg.br (A.M.C. Faria).

0008-8749/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.cellimm.2009.01.010

2.3. Immunization procedure

Animals were immunized intraperitoneally (i.p.) with 10 μg Ova plus 3 mg $Al(OH)_3$ in 0.2 ml of saline. Booster consisted of 10 μg Ova in 0.2 ml saline and it was given 14 days after immunization.

2.4. Analysis of serum immunoglobulin levels by ELISA

Levels of OVA-specific and total immunoglobulins were determined by ELISA. Briefly, 96-well plates (NUNC) were coated with 2 μg/well OVA or 0.1 μg goat anti-mouse UNLB antibody, in coating buffer, pH 9.8, overnight. Wells were washed and blocked with 200 μl of PBS contain 0.25% casein for 1 h at room temperature. Serum were added to the plate and incubated for 1 h at room temperature, plates were washed and then peroxidase-conjugated streptavidin goat anti-mouse or rat anti-goat (Southern Biotechnology) 1:150000 was added, and plates were incubated for 1 h at 37 °C. Color reaction was developed at room temperature with 100 μl/well of orthophenylenediamine (1 mg/ml), 0.04% H_2O_2 substrate in sodium citrate buffer. Reaction was interrupted by the addition of 20 μl/well of 2 N H_2SO_4. Absorbance was measured at 490 nm by an ELISA reader (Bio-Rad Model 450 Microplate Reader).

2.5. Lymphocyte proliferation assay

Spleens were removed for cell suspension preparation. Cells were washed twice with HBSS and red cells were lysed by exposure to water followed by addition of 10× PBS to the tube. Briefly, 1×10^6 cells/well were incubated at 37 °C, 5% of CO_2 for 24, 48 or 72 h with 100 μl/well of concavalin A-ConA solution (2, 4 and 8 μg/ml) or medium. After 72 h, 25 μl of tritiated thymidine [metil-^{3}H] (20 μCi/ml; sp ativ. 5 Ci/mmol) was added and left for 18 h. Cells were harvested by automatic *Cell Harvester* after 6–8 h. Filters disks were placed in tubes containing non-aqueous scintillation fluid and beta radioactivity determined using a beta radiation counter (Shimadzu). Results were expressed as c.p.m. (counts per minute).

2.6. ELISPOT assay

Spleens and bone marrow were removed, cell suspensions prepared using a tissue homogenizer, and gently centrifuged. Immunoglobulin-producing cells were counted by ELISPOT as described by Sedgwick (Sedgwick et al., 1983). Briefly, polyestyren microplates (NUNC, Roskild, Denmark) were coated with 2 μg/well of goat anti-mouse Ig (Southern Biotechnology) and left overnight at 4 °C. After 18 h, plates were washed with PBS and blocked with 200 μl PBS containing 0.25% casein for 1 h at room temperature. Cells were then incubated (100 μl/well) at 37° 5% CO_2 atmosphere for 4 h. Plates were washed (20–30 s) three times with Tween 0.05% for cell lyses. Biotinylated anti-mouse, 50 μl/well, (Ig – 1:20000; IgG – 1:20000 – *Southern biotechonology*; IgM – 1:4000; IgA – 1:4000 – *SIGMA*) were added and plates were incubated overnight at 4 °C. Next day, plates were washed 3 times and incubated with 50 μl/well of streptavidin (1:4000 – *SIGMA*). Plates were washed 5 times with PBS-Tween 0.05% for 5 m, plates were vortexed between washes. Then substrate BCIP-Sigma B-8503, diluted in buffer with agarose pre-heated at 44–46 °C (50 μl/well), was added to the plates. They were incubated at 37 °C for 1–8 h. Spots were counted and expressed as number of spots/10^6 cells.

2.7. Statistical analysis

Differences were determined by ANOVA when more than two groups were compared and by Student's *t*-test when only two groups were compared (young and old mice). A value of $p < 0.05$ was considered to be significant.

3. Results

3.1. Serum levels of OVA-specific Ig, IgG and IgA but not IgM were reduced in aged mice

To investigate the effect of aging in immune responses to a specific antigen young (8-week-old) and old (70-week-old) mice were i.p. immunized with 10 μg OVA + 3 mg $Al(OH)_3$. A booster with soluble Ova was given 14 days afterwards. Fig. 1 shows that specific IgM levels were not altered (Fig. 1C) while serum anti-OVA Ig, IgG and IgA levels were reduced (Fig. 1A, B and D) in aged mice when compared to young mice.

3.2. Serum levels of total Ig, IgG and IgA but not IgM antibodies were increased in aged mice

To investigate aging associated changes in the global pool of serum antibodies, we used 8-week-old and 100-week-old mice

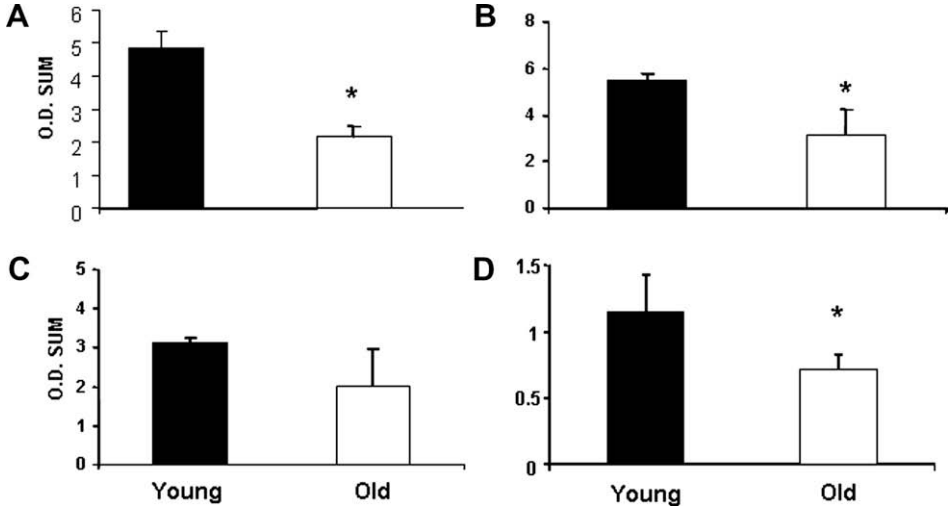

Fig. 1. OVA-specific serum Ig, IgG, IgM and IgA levels in young and old mice. OVA-specific serum Ig (A), IgG (B), IgM (C) and IgA (D) were measured by capture ELISA. Young mice (8-week-old) and old (70-week-old) were immunized i.p. with 10 μg OVA + 3 mg $Al(OH)_3$, a booster with OVA was given 7 days before serum collected. Bars represent the mean ± SD of 5 mice per group ($^*p < 0.05$). Data represent results obtained in two independent experiments.