Biogerontology
https://doi.org/10.1007/s10522-018-9774-4

RESEARCH ARTICLE

Social environment improves immune function and redox state in several organs from prematurely aging female mice and increases their lifespan

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Received: 20 July 2018 / Accepted: 19 September 2018

Abstract Aging is associated with a chronic oxidative stress (increase of oxidants and decrease of antioxidants), which contributes to immunosenescence and therefore shorter longevity. Nevertheless, a positive social network has been related to the adequate maintenance of health and deceleration of aging. Adult prematurely aging mice (PAM) are characterized by their inadequate stress response to a T-maze, showing premature immunosenescence and oxidative stress establishment. These impairments contribute to shorter life spans in comparison to exceptional non-PAM (ENPAM). However, it is not known whether these characteristics of PAM could be prevented by a positive cohabitation. Therefore, the aim of the present work was to determine if the premature immunosenescence and oxidative stress shown by PAM could be avoided by the cohabitation with ENPAM, increasing their life span. Female CD1 PAM and ENPAM were divided into three experimental groups: PAM controls, ENPAM controls and a social environment experimental group, containing in the same cage ENPAM and PAM (proportion 5/2, respectively). After 2 months, mice were sacrificed and spleen, thymus, liver and heart removed. Later, several immune functions as well as oxidative stress parameters were assessed in spleen and thymus leukocytes. Also, several oxidative stress parameters were analyzed in liver and heart. The results showed that PAM, after co-housing with ENPAM, had improved immune functions and redox balance in spleen and thymus leukocytes. This improvement of redox state was also observed in liver and heart. Furthermore, all these positive effects seem to be related to the increased life span of PAM.

Keywords Social environment · Prematurely aging mice · Immunosenescence · Oxidative stress balance · Lifespan

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Introduction

Aging is characterized by a general and progressive deterioration of all functions of the organism, especially those of the homeostatic systems such as the nervous, endocrine and immune systems. This leads to

Published online: 25 September 2018

the loss of homeostasis and consequently of health (Besedovsky and Del Rey 2007). This age-related decline seems to be associated with an imbalance between oxidant/inflammatory compounds and anti-oxidant/anti-inflammatory defenses, in favor of the first, which contributes to the establishment of chronic oxidative and inflammatory stress (De la Fuente and Miquel 2009). In this context, it has been proposed that immune cells could present an age-related loss in their capacity to regulate the oxidant and inflammatory compounds that they produce in order to carry out an immune response, increasing the chronic oxidative stress of the organism (De la Fuente and Miquel 2009; Vida et al. 2017). In addition, this oxidative stress has been suggested as one of contributors of the changes in the immune system produced by aging, which are known as immunosenescence (Salminen et al. 2008; Hazeldine and Lord 2015; Tu and Rao 2016; Weyand and Goronzy 2016). This impairment of immune function enhances the risk of suffering infections, autoimmune diseases and cancers, as well as increasing the probability of early death (Wayne et al. 1990; Ferguson et al. 1995; De Martinis et al. 2006). In fact, there is a relation between the redox state of the immune cells, the function capacity of these cells and the lifespan of the individual (De la Fuente et al. 2003; De la Fuente and Miquel 2009). Moreover, this oxidative stress observed in immune cells is also present in non-immunological tissues, as has been reported in chronologically old experimental animals (Vida et al. 2011) and in several models of rodents with accelerated aging (Takeda 2009; Baeza et al. 2010).

One of the characteristics of the aging process is its high heterogeneity. This explains the different rate of aging in individuals of the same chronological age, leading to the concept of biological age, which estimates the physiological state of each individual compared with others of the same chronological age. Thus, biological age is a better indicator than chronological age of the state of health, vitality and remaining life expectancy of each individual (Borkan and Norris 1980; Bulpitt et al. 2009). Although biological age is difficult to determine, several immune functions have recently been proposed as markers of this and as predictors of longevity (Martínez de Toda et al. 2016). In order to corroborate these markers and due to the high life expectancy of humans, the development of animal models of premature aging is very useful. In this context, our research group has developed a model of prematurely aging mice (PAM) based on the inadequate response shown by these animals when they are submitted to a T-maze test. These PAM show higher freezing and grooming behavior as well as slower locomotion than their counterparts of the same sex and chronological age [exceptional-non-PAM (ENPAM)]. For this reason, PAM exhibit an excess of reactivity to the stress generated by the maze, and therefore, the time to cross the intersection of the T-maze is increased. Furthermore, PAM, at adult age, showed a premature immunosenescence, which seems to be associated with higher oxidative stress, in comparison to ENPAM (Alvarado et al. 2006a, b; Viveros et al. 2007). All these alterations contribute to a shorter lifespan in the PAM group than their ENPAM counterparts, confirming a higher biological age in these animals (Guayerbas et al. 2002a, b, Guayerbas and De la Fuente 2003; Martínez de Toda et al. 2016).

Although the adequate maintenance of health depends on genetic background, growing evidence suggests that lifestyle could be a bigger influence (Johansson and Sundquist 1999; Molarius et al. 2007). Indeed, lifestyle strategies, such as nutrition and exercise, have been proposed as positive interventions capable of modifying the age-related impairments (De la Fuente et al. 2011). In social species, such as humans and rodents, the social context is essential for survival and reproductive success and provides protection from environmental threats. In fact, the existence of strong social networks may profoundly influence the physiological responses and positively correlate with health (Seeman and Crimmins 2001; Devries 2002). However, in this context, most reports have examined the negative effects of social environment. Thus, loneliness in humans and social isolation in rodents have been described as potential risk factors able to cause behavioral abnormalities and immune system impairments, resulting in a shorter lifespan (Bugajski 1999; Cohen et al. 2007; Cruces et al. 2014). Similarly, healthy rodents that cohabited with sick mice exhibited negative alterations in behavior and immune parameters (Morgulis et al. 2004; Palermo-Neto and Alves 2014). Moreover, healthy mice showed the induction of scratching and dermatitis after living with animals that presented this pathology (Hashimoto et al. 2006). In contrast, the existence of a positive social context has been related to longer

lifespan (Holt-Lunstad et al. 2010). In agreement with this, a previous preliminary study has demonstrated that premature immunosenescence in peritoneal leukocytes from tyrosine hydroxylase haploinsufficient mice (TH-HZ), a genetic alteration proposed as a premature aging model (Garrido et al. 2018b), may be avoided by cohabitation with wild type counterparts (Garrido et al. 2017). Nevertheless, the possible beneficial effects on immune system functions and oxidative stress in PAM have not yet been studied. Therefore, the aim of the present work was to determine if, in PAM, the premature immunosenescence and oxidative stress present in leukocytes of spleen and thymus as well as in other non-immunological tissues, could be avoided by a positive social context such as the cohabitation with ENPAM, as well as if this fact could affect their lifespan.

Materials and methods

Animals

One hundred female outbred CD1 mice (Janvier, France) between 32 and 33 weeks of age were purchased. They were housed seven to eight per cage and maintained with ad libitum access to food and tap water under light (12 h light/dark cycle; lights on at 8:00 a.m.), temperature (22 ± 2 °C) and humidity (50–60%) controlled conditions. Diet was in accordance with the recommendations of the American Institute of Nutrition for Laboratory Animals (A04 diet from Panlab S.L., Barcelona, Spain). The protocol was approved by the Experimental Animal Committee of the Complutense University of Madrid (Spain). Also, all mice were treated according to the guidelines of the European Community Council Directives 2010/63/EU. We used this strain due to its higher genetic heterogeneity than inbred strains. This could facilitate the extrapolation of the results to humans. Moreover, we have performed the majority of the studies with PAM and ENPAM using the CD1 strain (Guayerbas et al. 2002a; Guayerbas and De la Fuente 2003; Viveros et al. 2007; Martínez de Toda et al. 2016). We used female mice since this sex is more adequate to co-housing studies due to the fact that females do not show the aggressive and dominant behavior of males. Thus, it is more difficult to house males in groups and their behavior can interfere in the results obtained in the analysis carried out on parameters of the nervous and immune systems. Furthermore, these behaviors can alter the results, showing a different pattern in the case of male mice.

Selection of prematurely aging mice (PAM)

The mice, after 1 week of acclimatization in the animal room, were marked for their individual follow-ups and were submitted to a T-maze test. This test was performed once a week for four consecutive weeks to sort out the PAM (mice that cross the intersection of two arms in > 10 s in all four tests) from the ENPAM (which cross the intersection of two arms in < 10 s in all four tests) as previously reported (Guayerbas et al. 2002a; Martínez de Toda et al. 2016). Animals showing an intermediate response (approximately 65% of population), which we called "regular" mice, were removed from the study. This test was always performed under red light, between 09:00 and 11:00 h, to minimize circadian variations.

Experimental design

At age of 37–38 weeks, a group of 28 ENPAM and 16 PAM were randomly divided into the following groups: control ENPAM (C-ENPAM, N = 8), control PAM (C-PAM, N = 8), social environment ENPAM (SE-ENPAM) and social environment PAM (SE-PAM). These SE-PAM were PAM housed in cages with ENPAM (two PAM for each five ENPAM in each cage, the latter being denominated SE-ENPAM). This proportion was chosen in order to avoid possible interferences due to changes in the number of animals housed per cage, maintaining the same number as when they arrived at the laboratory. After 2 months of cohabitation under these conditions, mice were sacrificed by cervical dislocation according to the guidelines of the European Community Council Directives (2010/63/EU). Spleen, thymus, heart and liver were removed aseptically and freed from fat. In the case of spleen and thymus, each organ was minced with scissors and gently pressed through a mesh screen (Sigma-Aldrich, St Louis, USA). Spleen suspensions, due to their high concentration of erythrocytes, were centrifuged in a Ficoll-Hypaque (Sigma-Aldrich) gradient with a density of 1.070 g/mL. Cells from the interface were re-suspended in RPMI 1640 medium enriched with L-glutamine (PAA, Pasching,

Austria) and supplemented with 10% heat-inactivated fetal calf serum. The number of leukocytes was determined and adjusted to 10^6 cells/mL. Cellular viability, routinely measured before and after each experiment by the trypan-blue exclusion test, was higher than 95% in all experiments. All incubations were performed at 37 °C in a humidified atmosphere of 5% CO_2. The following studies were performed using unfractionated leukocytes to better reproduce the in vivo conditions of immune response and redox state. Another group of 28 ENPAM and 16 PAM were also randomly divided into the following groups: C-ENPAM (N = 8), C-PAM (N = 8), SE-ENPAM (N = 8) and SE-PAM (N = 8), and was used to analyze the possible beneficial effects of this strategy on their mean lifespan.

Immune function parameters

Chemotaxis

The chemotaxis capacity was carried out according to an original method previously described (Boyden 1962) with slight modifications introduced by us (Guayerbas et al. 2002a). Aliquots of 300 µL of leukocyte suspensions adjusted to 1×10^6 leukocytes/mL Hank's solution were added in the upper compartment of a chemotaxis chamber, and aliquots of 400 µL of the chemoattractant fMet-Leu-Phe (Sigma-Aldrich), at a concentration of 10^{-8} M, were placed in the lower compartment. This chemoattractant is characterized by affecting all kinds of leukocytes, so we the chemotaxis capacity can be evaluated in a similar way to in vivo conditions. The chambers were then incubated for 3 h and the filters were fixed and stained and the chemotaxis index (CI), as the number of leukocytes in the lower face of the filter, was obtained by counting, using an optical microscope. All CI were assayed in duplicate.

Natural Killer activity

In order to analyze the Natural Killer (NK) cytotoxic activity an enzymatic colorimetric assay (Cytotox 96 TM Promega, Germany) was used (Ferrández et al. 1999). Briefly, target cells (YAC-1 cells from a murine lymphoma) were seeded in 96-well U-bottom culture plates (Nunclon, Denmark) at a concentration of 10^4 cells/well in 1640 RPMI medium without phenol red. Leukocytes from spleen and thymus, as effector cells, were added at a concentration of 10^5 cells/well. Each sample was assayed in triplicate. The plates were then centrifuged at 250×g for 4 min to facilitate cell to cell contact, incubated for 4 h and centrifuged again. Lactate dehydrogenase activity was measured in supernatants by the addition of the enzyme–substrate at 490 nm. Three kinds of control measurements were performed: target spontaneous release, target maximum release, and effector spontaneous release. To determine the percentage of lysis of target cells, the following equation was used: $\% \text{ lysis} = [(E - ES - TS)/(M - TS)] \times 100$, where E is the mean of absorbance values in the presence of effector and target cells, ES the mean of absorbance values of effector cells incubated alone, TS the mean of absorbance values of target cells incubated alone, and M is the mean of maximum absorbance values after incubation of target cells with lysis solution. Results were expressed as percentages of lysis.

Lymphoproliferation

The proliferation of lymphocytes was assessed following a method previously described (Del Río et al. 1994). Spleen and thymus leukocyte suspensions were adjusted to a final concentration of 10^6 cells/mL in complete medium containing RPMI 1640 (PAA, Pasching, Austria), gentamicin (10 mg/mL, PAA) and 10% heat-inactivated fetal calf serum (PAA). Aliquots of 200 µL of spleen and thymus leukocytes were dispensed into 96-well plates (Nunc, Roskilde, Denmark). 20 µL/well of complete medium alone or supplemented with ConA (1 µg/mL, Sigma-Aldrich) or LPS (1 µg/mL, Sigma-Aldrich) were added. After 48 h of incubation at 37 °C in a sterile and humidified atmosphere of 5% CO_2, 100 µL of culture supernatant was removed and replaced by fresh medium. Then, 0.5 µCi [^{3}H] thymidine (MP Biomedicals, Santa Ana, CA, USA) was added to each well. The plates were incubated for an additional 24 h and finally, the cells were collected using a semi-automatic harvester (Skatron Instruments, Norway), and thymidine uptake was measured in a beta counter (LKB, Uppsala, Sweden) for 1 min. In the case of resting lymphoproliferation, the results were expressed as counts per minute (c.p.m.). Nevertheless, the lymphoproliferative responses to mitogens were calculated as the percentages of stimulation, 100% being the c.p.m.

obtained in the resting proliferation (wells without mitogens).

Oxidative stress parameters

Catalase activity

Catalase activity assay was carried out using a previously described protocol (Beers and Sizer 1952) with slight modifications introduced by us (Alvarado et al. 2006a, b). The enzymatic assay was followed spectrophotometrically for 80 s at 240 nm by the decomposition of H_2O_2 (14 mM in phosphate buffer) into $H_2O + O_2$. The results were expressed as international units (IUs) of enzymatic activity per 10^6 leukocytes or milligram of tissue.

Glutathione content

Both oxidized (GSSG) and reduced (GSH) forms of glutathione were evaluated using a fluorometrical technique previously described (Hissin and Hilf 1976) with slight modifications introduced by us (Garrido et al. 2017). This assay consists of the reaction capacity that GSSG presents with *o*-phthalaldehyde (OPT; Sigma-Aldrich), a fluorescent reagent, at pH 12, and the GSH at pH 8, resulting in the formation of a fluorescent compound. Spleen, thymus, heart and liver homogenates were re-suspended in phosphate buffer containing EDTA (0.1 M, pH 8, Sigma-Aldrich). Then samples were sonicated and after the addition of 5 µL of $HClO_4$ (60%, Sigma-Aldrich), they were centrifuged at 9500×g for 10 min at 4 °C. Aliquots of 10 µL of supernatants were dispensed into two 96-well black plates (Nunc), one for each glutathione form. For GSSG content, 8 µL of *N*-ethylmaleimide (NEM, 0.04 M, Sigma-Aldrich) was added to each well to prevent the interference of GSH with the measurement of GSSG and incubated at room temperature for 30 min in the dark. Following this, 182 µL of NaOH (0.1 N, Panreac) and 20 µL of OPT (1 mg/mL in methanol) were incorporated and the plate was incubated for 15 min under the same conditions. The fluorescence emitted by each well was measured at 350 nm excitation and 420 nm emission, and the results were expressed as nmol GSSG/10^6 leukocytes or nmol GSSG/mg tissue. For GSH content, 190 µL of phosphate buffer with EDTA and 20 µL of OPT was added to the 10 µL of supernatants dispensed in the wells. The plate was incubated for 15 min under the same conditions, and the fluorescence emitted by each well was measured at the same wave-length. The results were expressed as nmol GSH/10^6 leukocytes or nmol GSH/mg tissue. Also, GSSG/GSH ratios were calculated.

Xanthine oxidase activity

This oxidant enzyme was assayed using a commercial kit (A-22182 Amplex Red Xanthine/Xanthine Oxidase Assay Kit, Molecular Probes, Paisley, UK). This assay is based on the oxidation of purine bases (xanthine/hypoxanthine) to uric acid and superoxide anion by xanthine oxidase. The superoxide spontaneously degrades in the reaction mixture to hydrogen peroxide, which in the presence of horseradish peroxidase (HPR) reacts stoichiometrically with the Amplex Red reagent to generate the red-fluorescent oxidation product resorufin. In the assay, 50 µL of homogenates was incubated with 50 µL working solution of the Amplex Red reagent (100 µM) containing HPR (0.4 U/mL) and xanthine (200 µM). After 30 min of incubation at 37 °C, measurement of fluorescence was performed in a microplate reader (Fluostar Optima, BMG Labtech, Biomedal, Spain) using excitation and emission detection at 530 and 595 nm, respectively. The xanthine oxidase (10 mU/mL) supplied in the kit was used as the standard, and xanthine oxidase activity was measured by comparing the fluorescence of samples with that of standards. The results were expressed as units (U) of enzymatic activity per 10^6 leukocytes or milligram of tissue.

Mean lifespan

In order to evaluate the possible beneficial effects of cohabitation on mean lifespan, a group of SE-PAM, SE-ENPAM and their corresponding controls PAM and ENPAM, were housed in the same conditions until their natural death.

Statistical analysis

SPSS 21.0 (Chicago, USA) was used for the statistical analysis of the present results. All data were expressed as the mean ± standard deviation, each value being the mean of duplicate or triplicate assays. The normality of samples as well as homogeneity of

variances were evaluated by the Kolmogorov–Smirnov and Levene analyses, respectively. In the case of mean lifespan, the Kaplan–Meier test was used. Differences were studied through Student's test for independent samples. $p < 0.05$ was considered the minimum level of significance.

Results

Immune function in leukocytes from spleen and thymus

The results corresponding to immune function parameters evaluated in spleen and thymus leukocytes from C-PAM, SE-PAM, SE-ENPAM as well as C-ENPAM are summarized in Figs. 1 and 2, respectively.

The CIs, a function that represents the migration capacity of immune cells to a focus of infection, were lower in spleen and thymus leukocytes from C-PAM in comparison to those from C-ENPAM ($p < 0.001$). This fact also occurs with aging (De la Fuente et al. 1998; Martínez de Toda et al. 2016; Turner and Mabbott 2017). However, PAM after cohabitation with ENPAM (SE-PAM), exhibited greater chemotactic activity in both kinds of immune cells than C-PAM ($p < 0.001$; Figs. 1a, 2a). Nevertheless, although the values in SE-PAM were lower than in C-ENPAM ($p < 0.001$), they were similar to those obtained by ENPAM cohabiting with PAM (SE-ENPAM) in both kinds of immune cells. Furthermore, regarding the SE-ENPAM group, these animals showed lower spleen and thymus chemotactic capacity than C-ENPAM ($p < 0.001$). In the case of NK cytotoxic activity, shown as the percentage of lysis of tumor cells by spleen and thymus leukocytes, the C-PAM presented lower percentages than C-ENPAM ($p < 0.001$ and $p < 0.01$, respectively). This fact agrees with the age-related decrease of this function (De la Fuente et al. 1998; Martínez de Toda et al. 2016; Turner and Mabbott 2017). However, the SE-PAM group exhibited higher NK capacity in both types of immune organs than C-PAM ($p < 0.001$ and < 0.01, respectively; Figs. 1b, 2b). In this activity the SE-ENPAM group showed similar values to C-ENPAM.

With the objective of analyzing acquired immunity response, the percentages of stimulation of the lymphoproliferative responses to LPS and ConA have been analyzed in this work. The results are shown in Fig. 1c, d for spleen leukocytes, respectively, and Fig. 2c, d for thymus cells, respectively. As in the case of aged individuals (De la Fuente et al. 1998; Martínez de Toda et al. 2016; Turner and Mabbott 2017), spleen and thymus leukocytes from the C-PAM group showed lower values than those from C-ENPAM ($p < 0.001$). However, spleen and thymus leukocytes from SE-PAM presented higher values of both kinds of lymphoproliferations than the C-PAM group ($p < 0.001$). Although no differences were observed in the values of SE-ENPAM and C-ENPAM, the percentages in spleen and thymus leukocytes from SE-ENPAM being higher than those in C-PAM ($p < 0.001$ for LPS and ConA in spleen, and $p < 0.001$ for LPS and $p < 0.05$ for ConA in thymus).

Another immune feature that appears with aging as a consequence of the accumulation of inflammatory compounds together with a decrease of anti-inflammatory defenses, is the establishment of a sterile inflammation (De Martinis et al. 2006; Feldman et al. 2015). In this context, resting lymphoproliferation, which could be an immune function representative of this type of inflammation, was evaluated. The proliferation of spleen leukocytes without any stimulation, was higher in C-PAM than C-ENPAM ($p < 0.001$). However, SE-PAM and SE-ENPAM had lower resting lymphoproliferation in comparison to C-PAM ($p < 0.001$ and < 0.01, respectively; Fig. 1e). In the case of thymus leukocytes, although C-PAM showed higher resting lymphoproliferation than C-ENPAM ($p < 0.001$), no differences were observed between SE-PAM and SE-ENPAM with respect to C-PAM. Nevertheless, SE-PAM and SE-ENPAM had higher resting lymphoproliferation than C-ENPAM ($p < 0.01$ and < 0.001, respectively; Fig. 2e).

Thus, PAM after the cohabitation with ENPAM showed an improvement in all immune functions analyzed. This fact could represent a slowing down of immunosenescence in the spleen and thymus leukocytes of SE-PAM. However, SE-ENPAM, although they showed an impairment of the chemotaxis capacity of spleen and thymus leukocytes after the cohabitation with the PAM group, they did not show any changes with respect to C-ENPAM in the other functions studied.

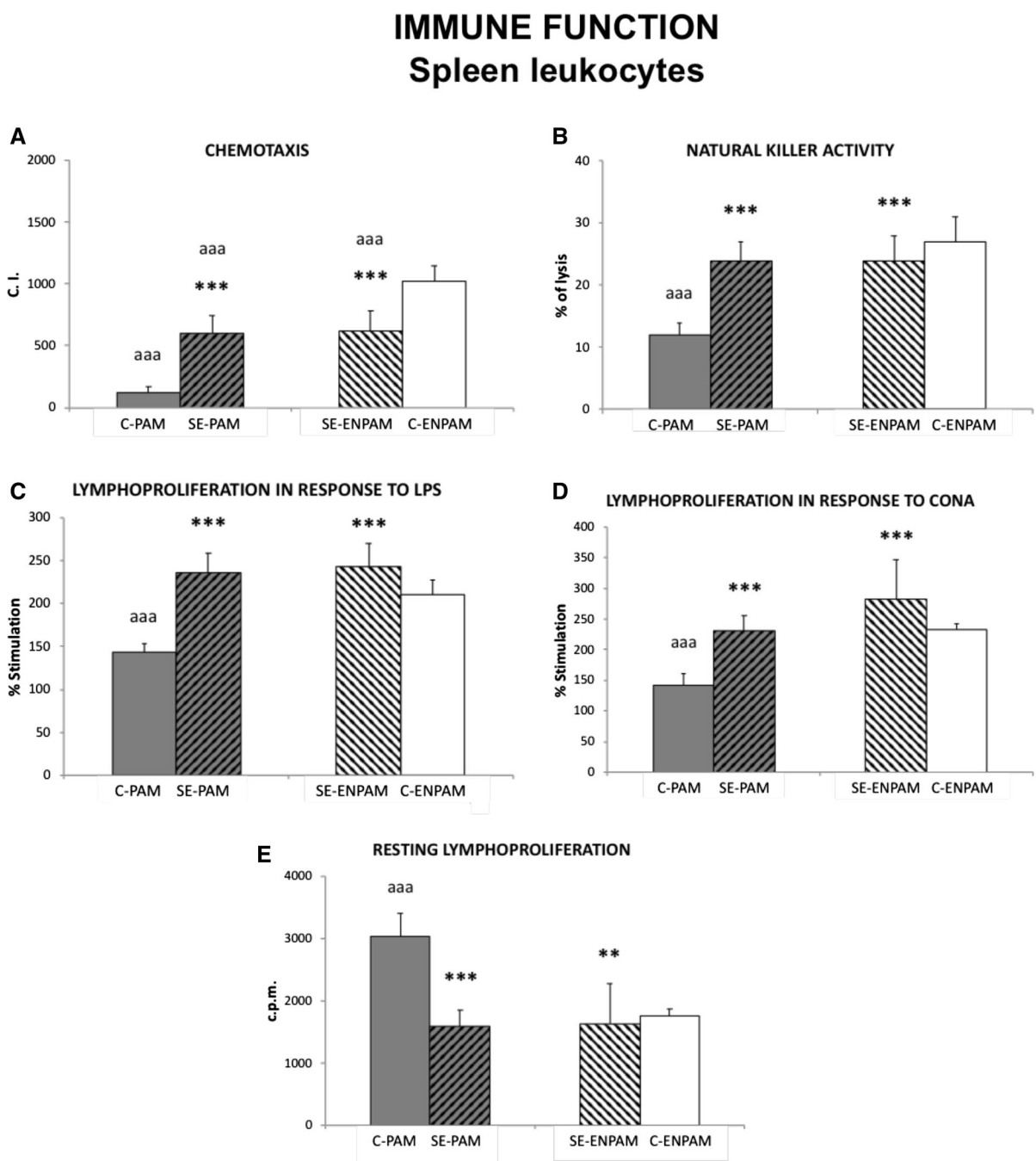
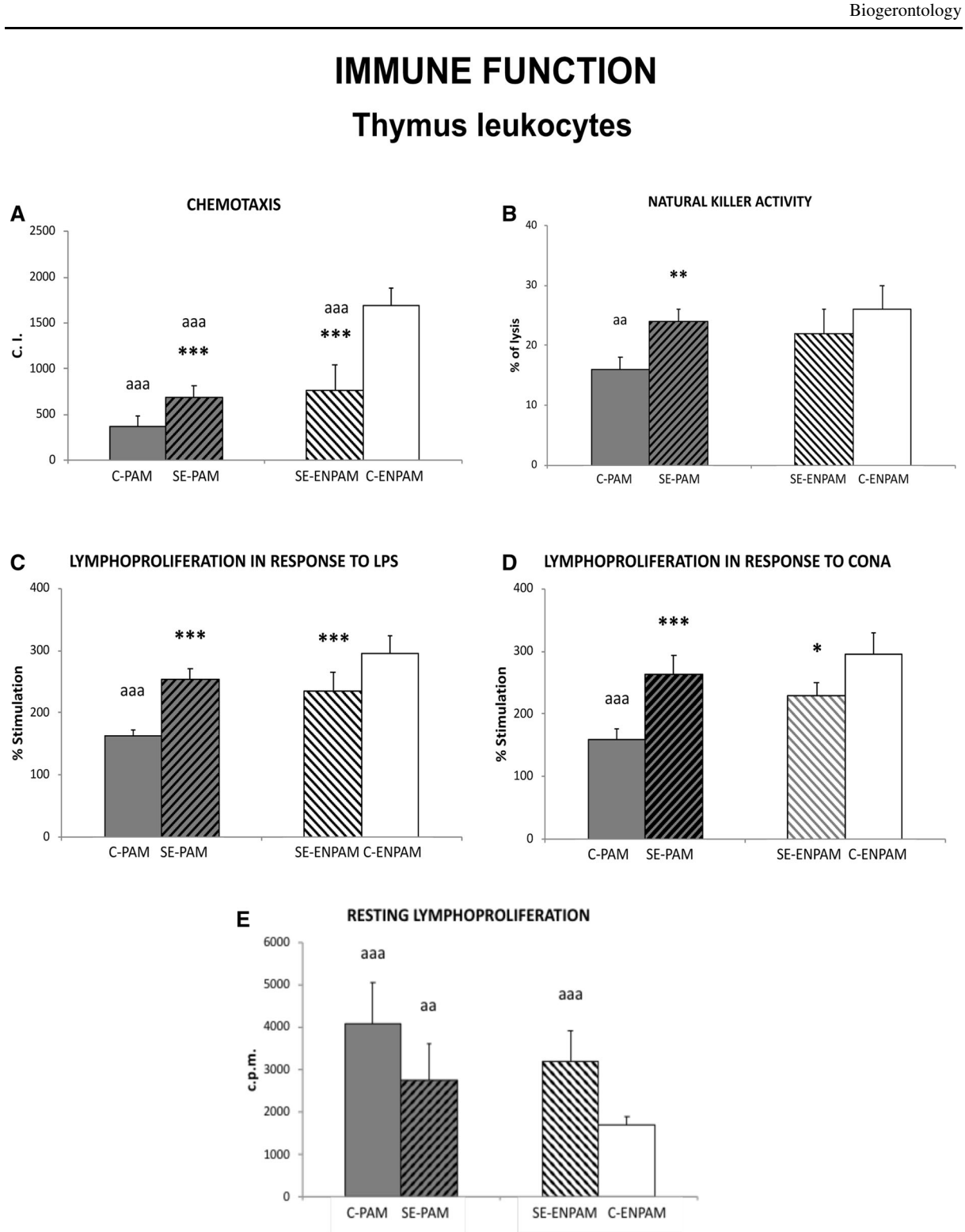


Fig. 1 Immune functions in spleen leukocytes. Chemotaxis index (CI, **a**), Natural Killer activity (% lysis of tumor cells, **b**), lymphoproliferative response to LPS (%, **c**), lymphoproliferative response to ConA (%, **d**) and resting lymphoproliferation (**e**) of spleen leukocytes. Each column represents the mean ± standard deviation of values corresponding to eight animals, with each value being the mean of duplicate or triplicate assays. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to C-PAM, $^{aa}p < 0.01$, $^{aaa}p < 0.001$ with respect to C-ENPAM. *C-PAM* control PAM, *SE-PAM* social environment PAM, *SE-ENPAM* social environment ENPAM, *C-ENPAM* control ENPAM


IMMUNE FUNCTION
Thymus leukocytes
A
CHEMOTAXIS
C. I.
2500
2000
1500
1000
500
0
aaa
aaa
***
aaa
***
C-PAM
SE-PAM
SE-ENPAM
C-ENPAM
B
NATURAL KILLER ACTIVITY
% of lysis
40
30
20
10
0
aa
**
C-PAM
SE-PAM
SE-ENPAM
C-ENPAM
C
LYMPHOPROLIFERATION IN RESPONSE TO LPS
% Stimulation
400
300
200
100
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C-PAM
SE-PAM
SE-ENPAM
C-ENPAM
D
LYMPHOPROLIFERATION IN RESPONSE TO CONA
% Stimulation
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*
C-PAM
SE-PAM
SE-ENPAM
C-ENPAM
E
RESTING LYMPHOPROLIFERATION
c.p.m.
6000
5000
4000
3000
2000
1000
0
aaa
aa
aaa
C-PAM
SE-PAM
SE-ENPAM
C-ENPAM

◀**Fig. 2** Immune functions in thymus leukocytes. Chemotaxis index (CI, **a**), Natural Killer activity (% lysis of tumor cells, **b**), lymphoproliferative response to LPS (%, **c**), lymphoproliferative response to ConA (%, **d**) and resting lymphoproliferation (**e**) of thymus leukocytes. Each column represents the mean ± standard deviation of values corresponding to eight animals, with each value being the mean of duplicate or triplicate assays. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ with respect to C-PAM, $^{aa}p < 0.01$, $^{aaa}p < 0.001$ with respect to C-ENPAM. *C-PAM* control PAM, *SE-PAM* social environment PAM, *SE-ENPAM* social environment ENPAM, *C-ENPAM* control ENPAM

Oxidative stress parameters in leukocytes from spleen and thymus

With advancing age, a chronic oxidative stress is established due to the decrease in anti-oxidant defenses together with the increase of pro-oxidant compounds (Rikans and Hornbrook 1997; Kasapoglu and Özben 2001). Since immune cell function is closely linked to the production of oxidant and anti-oxidant compounds and with aging there is an impairment of the regulatory capacity of the redox state (Salminen et al. 2008; De la Fuente and Miquel 2009; Vida et al. 2017), we analyzed several anti-oxidant defenses and oxidant compounds in both spleen and thymus leucocytes from C-PAM, SE-PAM, SE-ENPAM as well as C-ENPAM. The results are summarized Table 1 and illustrated in Fig. 3. As anti-oxidant defenses we evaluated catalase activity as well as reduced glutathione (GSH) contents and as oxidants compounds, xanthine oxidase activity as well as oxidized glutathione (GSSG) amounts. GSSG/GSH ratios were also calculated.

With respect to anti-oxidant defenses and in the case of catalase, spleen leukocytes from the C-PAM group exhibited lower values of this activity than those from C-ENPAM ($p < 0.001$). However, both SE-PAM and SE-ENPAM showed higher catalase activity, after cohabitation, in comparison to the C-PAM group values ($p < 0.05$ and < 0.01, respectively; Fig. 3a). Nevertheless, no differences were observed between SE-PAM, SE-ENPAM as well as C-ENPAM. In the case of thymus leukocytes, C-PAM had lower activity of this enzyme than C-ENPAM ($p < 0.01$), while thymus leukocytes from the SE-PAM group exhibited a higher value of catalase activity than the C-PAM group ($p < 0.05$; Fig. 3d). Regarding reduced glutathione (GSH) amounts evaluated in spleen leukocytes, no differences were observed between all experimental groups. Nevertheless, in the case of GSH contents analyzed in thymus leukocytes, higher GSH amounts were obtained in SE-PAM in comparison to C-PAM ($p < 0.05$; Table 1).

In relation to oxidant compounds, xanthine oxidase activity in spleen and thymus leukocytes from C-PAM was higher than in leukocytes from C-ENPAM ($p < 0.001$ and < 0.01, respectively). However, the SE-PAM group presented lower xanthine activity values in comparison to the C-PAM group ($p < 0.05$;

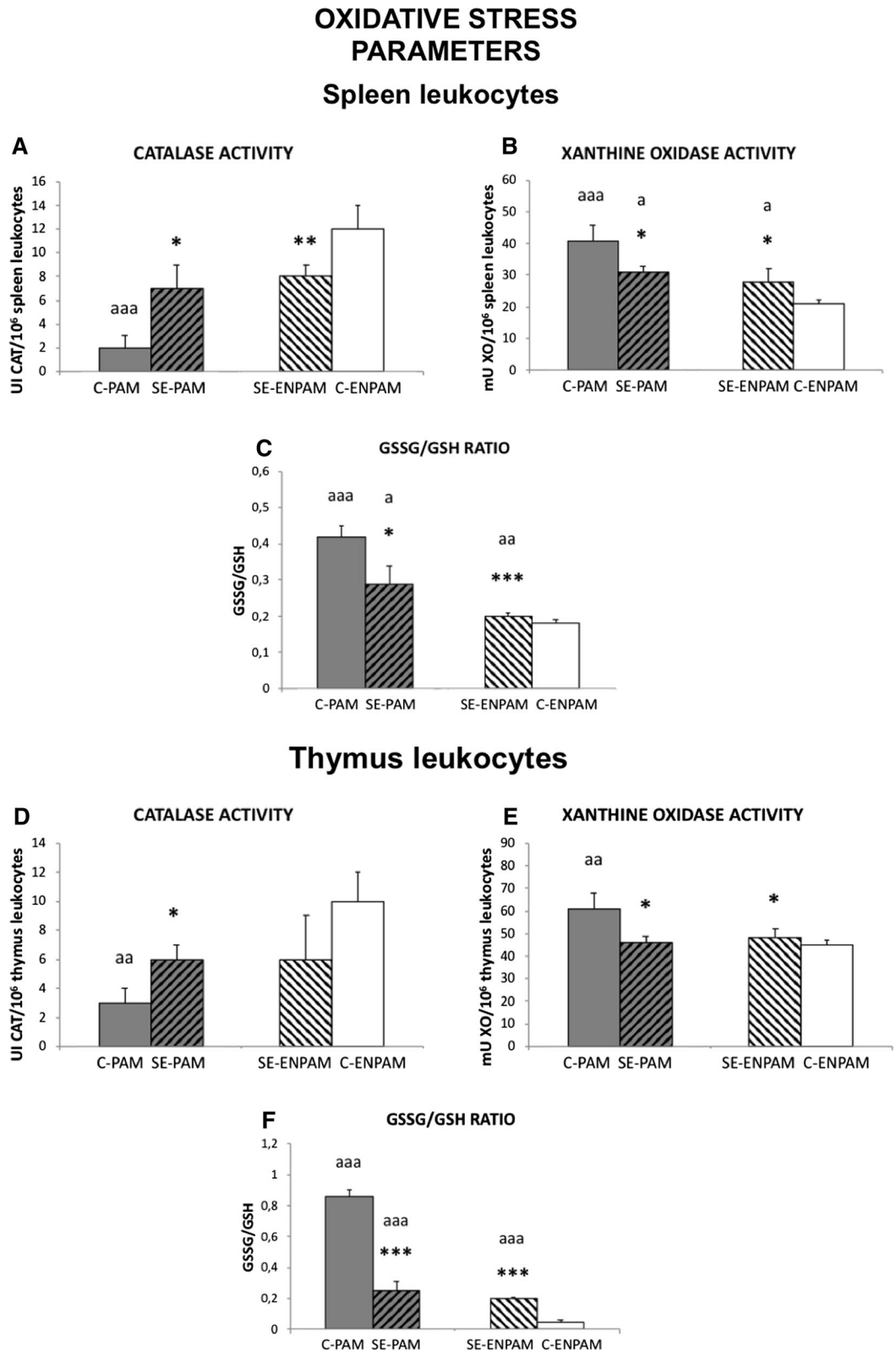
Table 1 Oxidative stress parameters in spleen and thymus leukocytes

	C-PAM	SE-PAM	SE-ENPAM	C-ENPAM
Spleen leukocytes				
Anti-oxidant defenses				
Reduced glutathione (GSH) (nmol GSH/10^6 spleen leukocytes)	12 ± 1	14 ± 3	12 ± 3	11 ± 3
Oxidant compounds				
Oxidized glutathione (GSSG) (nmol GSSG/10^6 spleen leukocytes)	5 ± 2	4 ± 1	4 ± 1	2 ± 1
Thymus leukocytes				
Anti-oxidant defenses				
Reduced glutathione (GSH) (nmol GSH/10^6 thymus leukocytes)	7 ± 1	12 ± 2*	10 ± 3	7 ± 2
Oxidant compounds				
Oxidized glutathione (GSSG) (nmol GSSG/10^6 thymus leukocytes)	6 ± 1^{aaa}	3 ± 1*^{aaa}	2 ± 1**^{aaa}	0.35 ± 0.09

Each value represents mean ± standard deviation of eight values corresponding to this number of animals

C-PAM control PAM, *SE-PAM* social environment PAM, *SE-ENPAM* social environment ENPAM, *C-ENPAM* control ENPAM

*$p < 0.05$, **$p < 0.01$ with respect to C-PAM, $^{aaa}p < 0.001$ with respect to C-ENPAM


OXIDATIVE STRESS PARAMETERS
Spleen leukocytes
A
CATALASE ACTIVITY
UI CAT/10⁶ spleen leukocytes
aaa
*
**
C-PAM SE-PAM SE-ENPAM C-ENPAM
B
XANTHINE OXIDASE ACTIVITY
mU XO/10⁶ spleen leukocytes
aaa
a
*
a
*
C-PAM SE-PAM SE-ENPAM C-ENPAM
C
GSSG/GSH RATIO
GSSG/GSH
aaa
a
*
aa
***
C-PAM SE-PAM SE-ENPAM C-ENPAM
Thymus leukocytes
D
CATALASE ACTIVITY
UI CAT/10⁶ thymus leukocytes
aa
*
C-PAM SE-PAM SE-ENPAM C-ENPAM
E
XANTHINE OXIDASE ACTIVITY
mU XO/10⁶ thymus leukocytes
aa
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C-PAM SE-PAM SE-ENPAM C-ENPAM
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GSSG/GSH RATIO
GSSG/GSH
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C-PAM SE-PAM SE-ENPAM C-ENPAM

◀**Fig. 3** Oxidative stress in spleen and thymus leukocytes. Catalase activity (IU CAT/10^6 leukocytes) of spleen (**a**) and thymus (**d**) leukocytes, xanthine oxidase activity (U XO/10^6 leukocytes) of spleen (**b**) and thymus (**e**) leukocytes, and GSSG/GSH ratios of spleen (**c**) and thymus (**f**) leukocytes. Each column represents the mean ± standard deviation of values corresponding to eight animals, with each value being the mean of duplicate or triplicate assays. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ with respect to C-PAM, $^{a}p < 0.05$, $^{aa}p < 0.01$, $^{aaa}p < 0.001$ with respect to C-ENPAM. *C-PAM* control PAM, *SE-PAM* social environment PAM, *SE-ENPAM* social environment ENPAM, *C-ENPAM* control ENPAM

Fig. 3b, e). Nevertheless, in the case of spleen leukocytes, the SE-PAM and SE-ENPAM groups had higher activity values for this enzyme than the C-ENPAM group ($p < 0.05$). In the case of oxidized glutathione (GSSG) contents, whereas no differences were observed between all experimental groups in leukocytes of spleen, whereas in the case of thymus leukocytes from C-PAM these had higher values than C-ENPAM ($p < 0.001$). In the case of SE-PAM and SE-ENPAM they showed lower GSSG contents than the C-PAM group ($p < 0.05$ and < 0.01, respectively). SE-PAM as well as SE-ENPAM presented higher amounts of GSSG with respect to C-ENPAM ($p < 0.001$; Table 1).

Finally, regarding GSSG/GSH ratios, a redox marker (Kand'ár 2016), the results obtained in spleen and thymus leukocytes are shown in Fig. 3c, f, respectively. While C-PAM had higher values for this ratio than C-ENPAM ($p < 0.001$), the SE-PAM group presented lower values in comparison to C-PAM ($p < 0.001$). However, SE-PAM and SE-ENPAM had higher values than C-ENPAM ($p < 0.05$ and < 0.01, respectively in spleen and $p < 0.001$ in thymus).

Oxidative stress parameters in liver and heart

The establishment of oxidative stress establishment affects all cells of the physiological systems (Salminen et al. 2008; De la Fuente and Miquel 2009; Vida et al. 2017). Since oxidative stress observed in immune cells can be reflected in other non-immunological tissues (Takeda 2009; Baeza et al. 2010; Vida et al. 2011), we wanted to determine whether this cohabitation could ameliorate or revert oxidative stress establishment in several non-immunological organs from PAM, analyzing the same anti-oxidant and pro-oxidant compounds previously evaluated in spleen and thymus leukocytes. Furthermore, there is a differential pattern in the organs depending on their capacity of division (Sohal et al. 1994; Hamilton et al. 2001). So, we chose liver, as a mitotic organ, and heart, as a post-mitotic organ in order to determine if these would be affected differently by this cohabitation. The results are summarized in Table 2 and Fig. 4.

Table 2 Oxidative stress parameters in liver and heart

	C-PAM	SE-PAM	SE-ENPAM	C-ENPAM
Liver				
Anti-oxidant defenses				
Reduced glutathione (GSH) (nmol GSH/mg tissue)	0.89 ± 0.18^{aa}	0.74 ± 0.07^{aaa}	1.22 ± 0.24	1.34 ± 0.12
Oxidant compounds				
Oxidized glutathione (GSSG) (nmol GSSG/mg tissue)	0.70 ± 0.60^{a}	0.59 ± 0.08	0.64 ± 0.08	0.54 ± 0.08
Redox index				
GSSG/GSH ratio	0.79 ± 0.09^{aaa}	0.79 ± 0.05^{aaa}	0.52 ± 0.07***	0.40 ± 0.06
Heart				
Anti-oxidant defenses				
Reduced glutathione (GSH) (nmol GSH/mg tissue)	0.31 ± 0.05	0.39 ± 0.08	0.27 ± 0.10	0.32 ± 0.04
Oxidant compounds				
Oxidized glutathione (GSSG) (nmol GSSG/mg tissue)	0.14 ± 0.04^{a}	0.15 ± 0.01^{aa}	0.10 ± 0.03	0.07 ± 0.01
Redox index				
GSSG/GSH ratio	0.45 ± 0.03^{aaa}	0.38 ± 0.02**^{aaa}	0.37 ± 0.03*^{aaa}	0.22 ± 0.04

Each value represents mean ± standard deviation of eight values corresponding to this number of animals

C-PAM control PAM, *SE-PAM* social environment PAM, *SE-ENPAM* social environment ENPAM, *C-ENPAM* control ENPAM

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ with respect to C-PAM, $^{a}p < 0.05$, $^{aa}p < 0.01$, $^{aaa}p < 0.001$ with respect to C-ENPAM

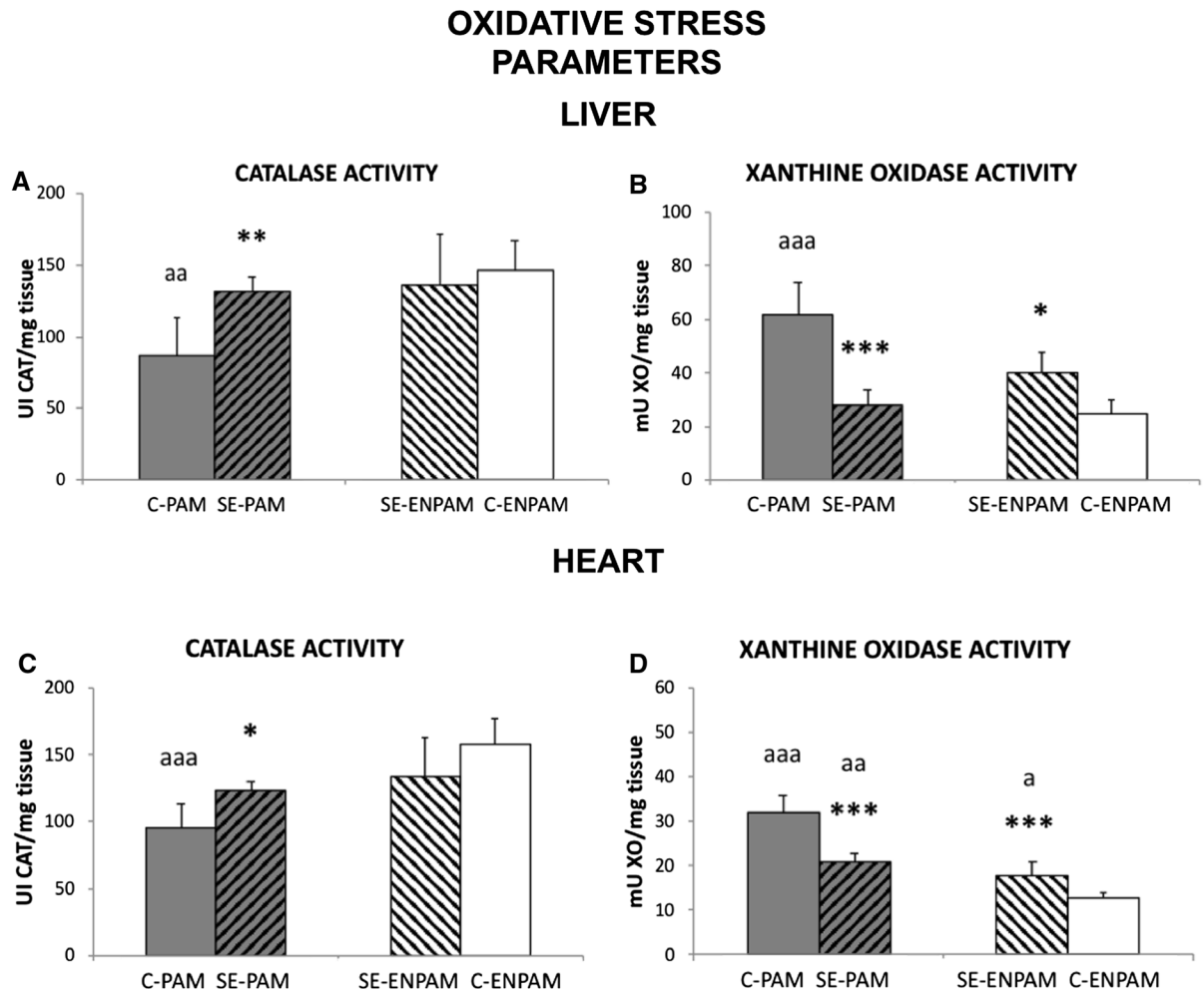

Fig. 4 Oxidative stress parameters in liver and heart. Catalase activity (IU CAT/mg tissue) of liver (**a**) and heart (**c**), xanthine oxidase activity (U XO/mg tissue) of liver (**b**) and heart (**d**). Each column represents the mean ± standard deviation of values corresponding to eight animals, with each value being the mean of duplicate or triplicate assays. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ with respect to C-PAM, $^{a}p < 0.05$, $^{aa}p < 0.01$, $^{aaa}p < 0.001$ with respect to C-ENPAM. *C-PAM* control PAM, *SE-PAM* social environment PAM, *SE-ENPAM* social environment ENPAM, *C-ENPAM* control ENPAM

The livers of C-PAM had lower catalase activity values than the C-ENPAM group ($p < 0.01$). However, SE-PAM presented higher activity values of this enzyme in comparison to C-PAM ($p < 0.05$; Fig. 4a). The same trend was observed in heart, showing lower catalase activity values in the C-PAM group than in C-ENPAM ($p < 0.001$), and higher values of this activity in SE-PAM in comparison to C-PAM ($p < 0.05$; Fig. 4c). In addition, regarding reduced glutathione (GSH) amounts evaluated in liver, C-PAM showed lower contents of this anti-oxidant defense than C-ENPAM ($p < 0.01$). No differences were observed in the other experimental groups, in both liver and heart (Table 2).

Regarding pro-oxidant xanthine oxidase activity analyzed in liver, while the C-PAM group had higher values than C-ENPAM ($p < 0.001$), the SE-PAM and SE-ENPAM groups presented lower activity values of this enzyme in comparison to C-PAM ($p < 0.001$ and < 0.05, respectively; Fig. 4b). Similarly, C-PAM also showed higher xanthine oxidase activity in heart than C-ENPAM ($p < 0.001$). However, SE-PAM had lower values in comparison to C-PAM ($p < 0.001$), while SE-PAM and SE-ENPAM presented higher values than C-ENPAM ($p < 0.01$ and < 0.05, respectively; Fig. 4d). With regards to oxidized glutathione (GSSG) amounts evaluated in liver, although C-PAM

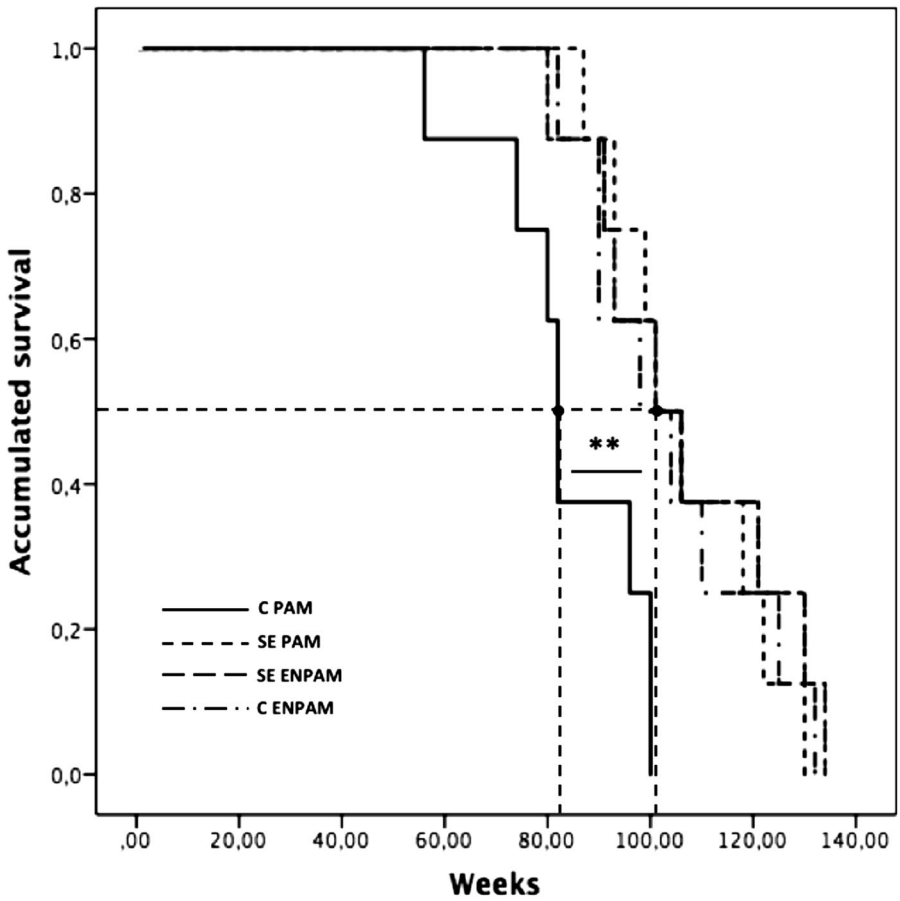

Fig. 5 Mean lifespan. Mortality records of C-PAM, SE-PAM, SE-ENPAM and C-ENPAM (N = 8) until the natural death of the animals. **$p < 0.01$ with respect to C-PAM

presented higher GSSG contents in comparison to C-ENPAM ($p < 0.05$), no differences were observed between the other experimental groups. However, in the case of heart, both C-PAM and SE-PAM groups presented higher GSSG amounts than C-ENPAM ($p < 0.05$ and < 0.01, respectively; Table 2).

Finally, with respect to GSSG/GSH ratios analyzed in liver and heart, the values of C-PAM were higher than those of C-ENPAM ($p < 0.001$). However, although the ratios in SE-PAM were also higher than in C-ENPAM ($p < 0.001$), in heart they were lower than in C-PAM ($p < 0.01$). In heart, the ratios of SE-ENPAM were higher than those of C-ENPAM ($p < 0.001$), although in both organs these values in SE-ENPAM were lower than in the corresponding C-PAM ($p < 0.05$ in heart and $p < 0.001$ in liver; Table 2).

These results could indicate that the improvement in redox state observed in spleen and thymus leukocytes from the SE-PAM group has also been observed in non-immunological organs of these animals, results that support the idea that this kind of cohabitation seems to diminish the chronic oxidative stress previously observed in PAM.

Lifespan

The mean lifespan from C-PAM, C-ENPAM, SE-PAM and SE-ENPAM was analyzed in separate experimental groups designed for this purpose. As shown Fig. 5, PAM after cohabitation with ENPAM (SE-PAM) showed a higher mean lifespan than C-PAM ($p < 0.01$), reaching similar values to those obtained in C-ENPAM. No differences were observed between C-ENPAM and SE-ENPAM.

Discussion

This work is the first in which immunosenescence in chronologically adult PAM has been decreased using co-housing with ENPAM. The redox state is closely

associated with the appearance of immunosenescence. For this reason, in the present work in addition to studying some functions in spleen and thymus leukocytes, several oxidative stress parameters were also analyzed in these cells as well as in non-immunological organs, such as liver and heart. The results showed significant positive effects of this cohabitation on the immune function and redox parameters studied in PAM, as well as producing a higher lifespan in this group.

The immune system suffers numerous age-related changes, which affect both innate and acquired immunity (Gruver et al. 2007; Salminen et al. 2008; Hazeldine and Lord 2015; Weyand and Goronzy 2016; De la Fuente and Bauer 2016). Innate immunity is the first line of defense against viral and bacterial infections, initiating an inflammatory response (Solana et al. 2012; Weinberger 2017). For this reason, we analyzed several innate immune functions, such as NK activity and chemotaxis capacity, which decrease with aging, as has been described in chronologically old mice (De la Fuente et al. 1998; Martínez de Toda et al. 2016) and humans (Fulop et al. 2004; Plowden et al. 2004; Solana et al. 2012). In accord with this, previous studies have reported this impairment in PAM at adult age (Guayerbas et al. 2002a, b), similar to that shown by C-PAM in the present work. However, PAM that co-housed with ENPAM (SE-PAM) exhibited a higher chemotaxis capacity as well as NK activity in the immune cells studied. In agreement with these results, a greater NK activity in blood has been described in older adults with a positive social context (Lutgendorf et al. 2005). Furthermore, rodents that live in a positive social environment exhibit faster wound healing (Detillion et al. 2004; Glasper and Devries 2005), a process closely linked to good innate immunity. Besides, other environmental strategies, which suppose a positive social and physically active life, such as environmental enrichment, have shown similar results. In fact, chronologically adult mice housed in an enriched environment show a higher NK cell activity in spleen in comparison to control animals (Benaroya-Milshtein et al. 2004). Moreover, peritoneal leukocytes from chronologically old mice that lived in an enriched environment showed a comparable improvement in these immune functions (Arranz et al. 2010a, b). In addition, the same results have been described in peritoneal leukocytes from chronologically old mice, which cohabited with chronologically adult mice (Garrido et al. 2018b). Also, an improvement in the functions of these cells from TH-HZ mice, which show a premature aging (Garrido et al. 2018a), was observed after co-housing with wild type animals (Garrido et al. 2017). The age-related decline of several innate immune functions, such as NK activity, has also been related to higher morbidity and mortality (De la Rosa et al. 2006; Arranz et al. 2010b). Thus, the improvement observed in the PAM group after co-housing with ENPAM may avoid this premature age-decline of innate immunity and could be associated with a higher lifespan.

With respect to acquired immunity, evaluated in this work by the percentage of lymphoproliferation in response to LPS and ConA, which also show an age-related decline (Pawelec et al. 1997; De la Fuente et al. 1998; Hallgren et al. 1998; Martínez de Toda et al. 2016), the data showed that C-PAM presented lower proliferation response values than ENPAM, in agreement with previous results (Guayerbas et al. 2002b; Martínez de Toda et al. 2016). Nevertheless, SE-PAM had a higher lymphoproliferative response to both mitogens than C-PAM. Thus, this kind of cohabitation may also improve this immune function. Other environmental strategies, such as environmental enrichment, also have similar beneficial effects on this function in peritoneal leukocytes from chronologically old mice (Arranz et al. 2010a, b). Previous reports have described comparable results in peritoneal leukocytes from chronologically old mice co-housing with chronologically adult mice (Garrido et al. 2018b) and in TH-HZ male mice with their wild type counterparts (Garrido et al. 2017). Furthermore, the maintenance of these lymphoproliferative responses has been described as predictive of low morbidity and mortality (Arranz et al. 2010b; Martínez de Toda et al. 2017) due to T-cells being considered extremely sensitive to immunosenescence (Miller 1996; Castle 2000; Pawelec et al. 2002). Thus, the improvement observed in these immune functions in PAM after co-housing with ENPAM, could be associated with higher lifespan. In addition, a higher inflammatory control has been related to positive social context (Saxton et al. 2011). In agreement with this, resting lymphoproliferation, which can indicate the establishment of sterile inflammation, a typical fact in aging (Feldman et al. 2015), showed lower values in SE-PAM. This adequate inflammatory

control, due to social environment, could be one of the possible reasons for the improvement of immune function in this experimental group. In this regard, a similar inflammatory control has been described in chronologically old mice co-housing with chronologically adult mice (Garrido et al. 2018b). Thus, this environmental strategy seems to suppose an improvement of both immunities in PAM, avoiding the immunosenescence that these animals present in spleen and thymus leukocytes.

As previously mentioned, the age-related decline that occurs in physiological systems and especially in the immune system, is closely associated to an imbalance between oxidant compounds and antioxidant defenses, in favor of the first, contributing to the establishment of chronic oxidative stress (Dröge 2003; De la Fuente and Miquel 2009; Vida et al. 2017). Indeed, previous studies have described that PAM, at adult age, show this redox imbalance in peritoneal leukocytes (Alvarado et al. 2006a, b), and the present work has shown a similar establishment in spleen and thymus leukocytes from C-PAM. Surprisingly, PAM after co-housing with ENPAM (SE-PAM) presented higher antioxidant defenses such as GSH contents and catalase activity in leukocytes than the C-PAM group. Increased antioxidant defenses, as activity of the potent antioxidant enzyme CAT, could be a key mechanism in preventing endogenous damage caused by oxidative burst and chronic inflammation, an improvement that could lead to long-term preserved immune cell function and longevity. In fact, increased CAT in transgenic mice can delay aging and has been found to extend lifespan (Cutler 2005; Enns et al. 2008). Furthermore, the SE-PAM group showed lower GSSG contents in thymus leukocytes and lower xanthine oxidase activity in both kinds of cells. These results may indicate an adequate redox balance in PAM that cohabited with ENPAM. In fact, spleen and thymus leukocytes from these animals showed lower GSSG/GSH ratios in comparison to the C-PAM group, a parameter considered a marker of oxidative stress (Kand'ár 2016). Similar results have been described in peritoneal leukocytes from old animals living in an enriched environment (Arranz et al. 2010a, b), as well as in chronologically old mice co-housing with adult mice (Garrido et al. 2018b), and in these cells from TH-HZ mice cohabiting with wild animals (Garrido et al. 2017). The chronic oxidative stress that appears with aging is also reflected in non-immunological tissues, as previously described in chronologically old mice (Vida et al. 2011) and in several rodent models of accelerated aging (Takeda 2009; Baeza et al. 2010). In the present study, we have also observed this feature in liver and heart from C-PAM. Nevertheless, we have not observed the different pattern in oxidative stress parameters in these organs depending on their mitotic capacities, as has previously been described (Sohal et al. 1994; Hamilton et al. 2001). These differing results could be due to the premature aging shown by the PAM group. In fact, aging-associated changes in progenitor cell activation and in the stem cell niches, have been related to the loss of repair capacity in mitotic organs, such as liver. In agreement with this, previous studies have described that oxidative stress associated with aging inhibits liver progenitor cell activation in chronologically old mice, features that could occur in the PAM control group (Cheng et al. 2017). However, when PAM co-housed with ENPAM (SE-PAM), their liver and heart showed higher catalase activity and lower xanthine oxidase activity in comparison to C-PAM. These results seem to indicate that cohabitation with ENPAM avoids the chronic oxidative stress that PAM exhibit at adult age. Moreover, since immunosenescence is closely associated to chronic oxidative stress (Salminen et al. 2008; De la Fuente and Miquel 2009; Hazeldine and Lord 2015), this improvement in redox state could be another possible reason for the avoidance of the immunosenescence shown by SE-PAM.

By contrast, SE-ENPAM had higher oxidants (xanthine oxidase activity in spleen leukocytes as well as GSSG content in thymus leukocytes) than C-ENPAM, exhibiting an oxidative stress establishment due to their cohabitation with PAM. In fact, GSSG/GSH ratios, a redox marker (Kand'ár 2016), were higher in both kinds of cells from SE-ENPAM than from C-ENPAM. Similar results have been described in peritoneal leukocytes from chronologically adult mice cohabiting with chronologically old mice (Garrido et al. 2018b) as well as in adult wild type co-housing with TH-HZ mice (Garrido et al. 2017). Moreover, this oxidative stress was also observed in a post-mitotic organ such as heart of SE-ENPAM, with higher xanthine oxidase activity as well as higher GSSG/GSH ratios than in this organ from C-ENPAM. Furthermore, these results could be due to psychological stress produced by cohabitation with PAM. In fact, previous studies have reported that

cohabitation of healthy individuals with sick animals can produce a psychological stress (Morgulis et al. 2004; Palermo-Neto and Alves 2014), which has been related to an increased oxidative stress (Moller et al. 1996; Irie et al. 2001). Since immunosenescence is closely associated to oxidative stress, as commented above (Salminen et al. 2008; De la Fuente and Miquel 2009; Hazeldine and Lord 2015), the redox imbalance observed in SE-ENPAM after cohabitation could be responsible for the inadequate immune function in these animals. Previous results in chronologically adult mice that have co-housed with chronologically old mice (Garrido et al. 2018b) and in adult wild type mice cohabiting with TH-HZ animals (Garrido et al. 2017) support the same idea. Unexpectedly, ENPAM that cohabited with PAM (SE-ENPAM) only showed a lower chemotaxis capacity than C-ENPAM in both kinds of immune cells. These results could be due to several reasons, such as the chronological age of these animals (adults) as well as the characteristics of this group. It is necessary to consider that these ENPAM are adults with an excellent response to stress situations, better than that in regular adults, which is related to their longer lifespans (Martínez de Toda et al. 2018). This appropriate characteristic could suppose the development of hormetic mechanisms by these ENPAM due to this mild-stress exposure and explain the maintenance of their immune function. In agreement with this, several previous studies have related the beneficial long-term effects after mild-stress exposure, which can even lead to the development of a resilience capacity (Russo et al. 2012; Beery and Kaufer 2015). Additionally, high risk of suffering infectious diseases, autoimmune processes and cancers as well as the increasing probability of early death have been related to the establishment of immunosenescence (Wayne et al. 1990; Ferguson et al. 1995; De Martinis et al. 2006). Recently, several immune functions analyzed in this work have been proposed as markers of health and predictors of longevity (Martínez de Toda et al. 2016). In agreement with this, a stronger relation between positive social context and health has previously been reported, implying an increased lifespan (Seeman and Crimmins 2001; Uchino 2006). In fact, the SE-PAM group showed an increased mean lifespan, results that seem to support this idea. In this context, chronologically old mice that live with chronologically adult animals also show a higher mean longevity (Garrido et al. 2018b), exhibiting a similar positive effect. Thus, this strategy is capable of avoiding premature immunosenescence and recovering the redox balance from PAM, so increasing their mean lifespan.

Therefore, considering all results presented in this work, the co-housing with ENPAM seems to be a positive social environment for PAM, which produces beneficial effects on several immune function and redox parameters. Even though we are not certain as to how and why this cohabitation supposes a positive social environment, we hypothesized that the improvement could be due to the physiological characteristics of both experimental groups. The PAM group, although chronological adults, consisted of individuals with premature aging. This could have been associated with age-related impairments, such as a decline in social communication. In fact, changes in behavior, odor and vocalizations have been observed in chronologically old animals (Guan and Dluzen 1994; Osada et al. 2003; Salchner et al. 2004; Finkel et al. 2006). Furthermore, data not published seem to indicate that PAM show an altered social behavior. However, ENPAM are made up of exceptional adult individuals, which show an adequate social response (data not published). Thus, the cohabitation in an environment predominantly formed by ENPAM could cause PAM to develop mechanisms for improving social communication. This improvement may be due to visual, olfactory and auditory perceptions of the presence of ENPAM, which enhances the regulatory systems. Another possible mechanism could be the ingestion of fecal bolei, which may alter the microbiota of the PAM group (Foster et al. 2017). In fact, several previous studies have reported that co-housing of mice produces an exchange of microbiota. This process also occurs across different strains, showing a gastrointestinal tract synchronization, which is generated by coprophagy (Deloris et al. 2006; Hufeldt et al. 2010; Hildebrand et al. 2013). A previous report has described a trend to a lower presence of the *Bifidobacterium* group in distal colon content from PAM in comparison to ENPAM (De Palma et al. 2014). This feature also occurs with aging (Tiihonen et al. 2010; De Palma et al. 2014; Kumar et al. 2016; Maffei et al. 2017). These PAM, during cohabitation, could ingest fecal bolei from their ENPAM counterparts, increasing and diversifying their intestinal microbiota. Regarding this, the role of microbiota as a potential modulator of physiological systems, especially

nervous and immune systems, has been suggested (Diaz Heijtz et al. 2011; Geuking et al. 2014; Sharon et al. 2016). Thus, a possible recovery of microbiota populations could modulate immune functions and be associated with the improvement observed in the PAM group. Nevertheless, further experiments are needed in order to clarify and corroborate these hypotheses.

Interestingly, PAM are detected by their inappropriate response to stress situations, such as the exploration of a new environment, like a T-maze and characterized by their immunosenescence together with the establishment of oxidative stress, impairments that contribute to a shorter life span (Guayerbas et al. 2002a, b; Martínez de Toda et al. 2016). Similarly, a previous report has described that TH-HZ mice, which show an altered catecholaminergic homeostasis because of *th* gene depletion, exhibit an impairment of immune function and redox imbalance, changes associated with their lower life span (Garrido et al. 2018a, b). In fact, an inadequate stress response has been related to chronological, premature and accelerated aging (Viveros et al. 2007; Bauer 2008; Gouin et al. 2008; Cruces et al. 2014), and both premature aging models seem to support this idea. However, these animals after co-housing with ENPAM or WT, respectively, exhibited an improvement of immune function and oxidative stress parameters. These results seem to indicate the importance of this environmental strategy in the context of stress response. In fact, PAM after cohabitation, presented an improvement of all immune function and redox parameters analyzed in this work, supporting the idea that this social context is capable of ameliorating the consequences of an inappropriate stress response.

In conclusion, this work suggests that cohabitation of chronologically adult PAM with chronologically adult ENPAM produces, in the former, an improvement of several immune functions of spleen and thymus leukocytes. This cohabitation also causes the recovery of the redox state in these immune cells as well as in other non-immunological organs, such as liver and heart in PAM. All these positive effects produce an increased mean lifespan in this group. Nevertheless, this social strategy seems to have bidirectional effects, producing an increased oxidation in ENPAM, although this does not suppose an altered immune function and lower lifespan in these animals. Since this work constitutes the first attempt to analyze the effects of this environmental strategy on PAM, further research is needed in order to corroborate both the beneficial and prejudicial effects of this approach in other immune locations, such as peritoneal leukocytes from PAM and ENPAM, as well as to clarify the possible underlying mechanisms.

Funding This work was supported by the Grants of the Research Group of UCM (910379) and FIS (PI15/01787) from the ISCIII-FEDER of the European Union.

Compliance with ethical standards

Conflicts of interest The authors declare no have conflict of interest.

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