Experimental Gerontology 46 (2011) 462–467

Contents lists available at ScienceDirect

Experimental Gerontology

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

CpG-ODN + IFN-γ confer pro- and anti-inflammatory properties to peritoneal macrophages in aged mice

Miriam V. Liscovsky, Romina P. Ranocchia, Diego O. Alignani, Carolina V. Gorlino, Gabriel Morón, Belkys A. Maletto, María C. Pistoresi-Palencia *

Centro de Investigación en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

ARTICLE INFO

Article history:
Received 20 August 2010
Received in revised form 20 December 2010
Accepted 31 January 2011
Available online 22 February 2011

Section Editor: Dr. R. Effros

Keywords:
Aging
Arginase
CpG-ODN
IL-12
Macrophages
Nitric oxide

ABSTRACT

Aging is accompanied by a disturbance in the homeostasis of the immune system. However, research into the behavior of macrophages in aging has shown disagreements about the functional status of these cells in aged mice. In this work, we studied the influence of aging on macrophage functions by evaluating the pro- and anti-inflammatory parameters of peritoneal macrophages preserved in their natural microenvironment.
Resident peritoneal macrophages from old mice, in the context of their natural milieu, were found to respond with a similar phenotype and functional pattern to macrophages from young mice.
In addition, we evaluated the macrophage response to CpG-ODN, a well-known Th1 promoter. CpG-ODN + IFN-γ were able to activate not only nitric oxide to initiate the inflammatory response, but also IL-12 in resident and inflammatory peritoneal macrophages from aged mice in the context of their natural milieu, although some quantitative differences were found in IL-10 and IL-12 secretion. With this stimulus, NO secretion and arginase activation were maintained in peritoneal macrophages during aging. These results will help to elucidate potential immunization strategies with CpG-ODN in the elderly.

1. Introduction

Macrophages (MØ) are present in every tissue in the body and provide innate immune surveillance. Their activation induces biochemical and morphological modifications that permit their functional activities to be performed. On a functional basis, MØ have been broadly classified into two phenotypes: classically or alternatively activated MØ. Classical activation is induced under a Th1-like cytokine milieu or upon recognition of PAMPs, whereas alternative activation develops under the presence of Th2 cytokines (such as IL-10 and TGF-β), immune complexes, hormones, and apoptotic cells (Goerdt and Orfanos, 1999; Gordon, 2003; Mills et al., 2000; Mosser, 2003). These phenotypes have been related to pro- or anti-inflammatory properties, respectively. Nowadays, however, condensing MØ heterogeneity into just two subdivisions may be an oversimplification. It has been proposed that, in response to changes in their tissue environment, MØ can reversibly and progressively change the pattern of functions that they express (Stout and Suttles, 2004, 2005).

Some other reports have focused on evaluating the MØ function during aging. While many of the research teams have reported a decline in MØ function with age, others have demonstrated the opposite. Moreover, some others have found no effect of age (Kohut et al., 2004). For example, several studies have shown decreased chemotaxis and phagocytosis in MØ from aged humans and mice, but other reports using aged rats have found opposing results or even no age-related changes (Corsini et al., 2005; De La Fuente, 1985; Fietta et al., 1993; Hilmer et al., 2007; Miller et al., 2007). The discrepancies in these results may reflect differences in the activation state of the MØ, in their source, or in the particular experimental conditions.

The MØ-family cells have marked phenotypic heterogeneity, are present in every tissue in the body and are responsive to many endogenous and exogenous stimuli that induce distinctive programs of gene expression. In addition, while it has been suggested that some intrinsic aspects of MØ change with age, the microenvironment occurring in the aged subject probably plays a key role in defining the functionality and activation properties of MØ in response to different stimuli. The overproduction of mediators *in vivo* might also be compounded by the presence of other stimuli in the aged host (including hormones, cytokines, chemokines, adrenergic and cholinergic agonists, fatty acids and immunoglobulins), which are capable of impacting functional and

Abbreviations: MØ, Macrophages; CpG-ODN, oligodeoxinucleotides containing unmethylated CpG motifs; r-PC, resident peritoneal cells; PEC, peritoneal exudate cells; PAMPs, Pathogen-associated molecular patterns.

* Corresponding author at: CIBICI, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, (5000), Córdoba, Argentina. Tel.: +54 351 4344973/76; fax: +54 351 4333048.
E-mail address: cpistore@fcq.unc.edu.ar (M.C. Pistoresi-Palencia).

0531-5565/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.exger.2011.01.006

phenotypic MØ characteristics. Thus, the effect of the aged microenvironment has to be considered.

It has been previously observed that aged mice manifest a marked Th2 immune response bias, which could direct MØ activation towards an anti-inflammatory pattern (Maletto et al., 2005; Shearer, 1997). However, there is insufficient data about the way aging may affect the MØ anti-inflammatory response. Therefore, in the present work, we evaluated the effect of oligodeoxinucleotides containing unmethylated CpG motifs (CpG-ODN) on peritoneal MØ from old animals, and analyzed different aspects of their pro- and anti-inflammatory responses in their own natural milieu in order to evaluate MØ capacity in conditions similar to those found *in vivo*.

2. Materials and methods

3-month-old (young) and 18-month-old (old) female BALB/c mice were obtained from the Comisión Nacional de Energía Atómica (CNEA, Buenos Aires, Argentina) and the Bioterio Facultad de Ciencias Veterinarias (Universidad Nacional de la Plata, Argentina). They were maintained in our animal facilities, which met the terms of the Guide to the Care and Use of Experimental Animals, published by the Canadian Council on Animal Care (with the assurance number A5802-01 being assigned by the Office of Laboratory Animal Welfare (NIH)). Our Institutional Experimentation Animal Committee (authorization # 15-07-62010 and HCD resolution 450/07) also approved the animal handling and experimental procedures.

Mouse rIFN-γ was obtained from R&D Systems (Minneapolis, MN, USA). L-arginine hydrochloride, alpha-isonitrosopropiophenone (ISPF), sulphanilamide and naphthylethylene-diamine dihydrochloride were purchased from Sigma-Aldrich, Argentina S.A (Buenos Aires, Argentina). The CpG-ODN used was 1826 (TCCATGACGTTCCTGACGTT) (Operon Technologies, Alameda, CA, USA).

Mice were sacrificed and resident Peritoneal Cells (r-PC) were harvested by lavages with sterile cold Hanks' balanced salt solution (HBBS), pH 7.4, plus 100 μg/ml penicillin and 100 U/ml streptomycin (Invitrogen Argentina, Buenos Aires, Argentina). PEC were obtained by injecting i.p. 1 ml of 4% sterile solution of Brewer's thioglycollate medium (Laboratorios Britania S.A., Buenos Aires-Argentina) five days before harvesting the PEC.

Peritoneal cells were cultured in RPMI 1640 without phenol red medium (Sigma-Aldrich) supplemented with heat-inactivated FCS (Natocor, Córdoba, Argentina), 2 mM L-glutamine, 50 μM 2-mercaptoethanol (Sigma-Aldrich), 100 μg/ml penicillin and 100 U/ml streptomycin at 37 °C in a moist atmosphere of 5% CO_2 in air. Unless otherwise mentioned, 0.5 ml of r-PC suspension at 3×10^6 cell/ml or 0.5 ml of PEC suspension at 1×10^6 cell/ml were seeded in 48-well tissue culture plates (GREINER Bio One, Frickenhausen, Germany) for 48 h.

Arginase activity was measured in lysates from adherent cells as described by Corraliza et al. (Corraliza et al., 1994) with a few modifications (Liscovsky et al., 2009). Briefly, cells were lysed with 0.1% Triton X-100 plus protease inhibitors for 30 min. Equal volumes of 25 mM Tris–HCl–10 mM MnCl2 and cell lysate were mixed, and the enzyme was activated for 10 min at 55 °C. Arginine hydrolysis was performed by incubating the lysates with L-arginine (pH 9.7) at 37 °C for 60 min. This reaction was then stopped with 400 μl of $H_2SO_4/H_3PO_4/H_2O$ (1/3/7, v/v/v). The urea concentration was measured at 540 nm after the addition of 25 μl of ISPF (dissolved in 100% ethanol), before heating at 100 °C for 40 min. The values obtained (expressed as units of activity) were normalized against total protein (U arginase/mg protein) content in adherent cells. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol urea/min.

NO production was determined by measuring NO_2^- in cell supernatants using the Griess reagent. Briefly, 100 μl of culture supernatant were mixed with 200 μl of reagent (1% sulfanilamide/0.1% naphthylethylene diamine dihydrochloride/2.5% H_3PO_4) at room temperature for 10 min, after which the absorbance at 540 nm was determined.

The levels of IL-10 and IL-12 were measured in culture supernatants by capture ELISA, following the manufacturer's recommendations (BD Becton Dickinson Argentina S.R.L., Buenos Aires, Argentina). The following antibodies were used for coating and detection, respectively: JES5-2A5 and JES5-E16E3 for IL-10, and C15.6 and C17.8 for IL-12. Concentrations were expressed using standard curves. For detection of intracellular cytokine, we used BD Cytofix/cytoperm kit (BD) in accordance with the manufacturer's protocols and incubated with anti-mouse IL-12 antibody (clone C15.6) or an isotype control antibody (BD).

For flow cytometry, the cells were pretreated with CD16/CD32 (clone 2.4G2) and subsequently stained with the following antibodies from BD: anti-mouse-CD3 (clone 145–2C11), CD11c (clone HL3), CD19 (clone 1D3), CD23 (clone B3B4), CD80 (clone 16-10A1), CD86 (clone GL1), MHC-II (clone 2G9), CD5 (clone 53–7.3), CD11b (clone M1/70-5C6), and Gr-1 (clone RB6-8C4). Anti SR-A (clone 2f8), MMR (clone 5D3) and F4/80 (clone CI:A3-1) were obtained from Serotec (Oxford, UK). The WinMDI software, Version 2.9 (Joseph Trotter©, The Scripps Research Institute, La Jolla, CA, USA: http://facs.scripps.edu/) was used.

No differences were observed in cell size or complexity between r-PC from young and old mice.

Data were analyzed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA), a two-way ANOVA, followed by Bonferroni post-test for multiple comparisons, and the Student's *t* test for two groups. All data were considered statistically significant if p values were <0.05.

3. Results and discussion

In order to study the effect of aging on peritoneal cells, we first determined the cell populations present in old mice peritoneum. We found that peritoneum from old mice contained more r-PC than

Table 1
Cell populations in the peritoneum of native and thioglycollate injected young and old mice.

	Absolute number of cells ($\times10^6$)			Relative frequency of cellular populations in r-PC (%)				
	Total cells	Mϕ	B cells	Mϕ	B cells	T cells	Neutrophils	DCs
Y	5.4 ± 1.9	1.9 ± 1.2	2.8 ± 1.2	35 ± 13	52 ± 9	3.1 ± 1.9	0.8 ± 0.4	1.3 ± 0.8
O	11.6 ± 4.7**	1.5 ± 0.8	9.3 ± 4.9**	14 ± 8*	75 ± 9*	3.8 ± 1.6	0.5 ± 0.4	0.6 ± 0.3

*p<0.01; **p<0.0001 compared to the young counterpart.

	Absolute number of cells ($\times10^6$)			Relative frequency of cellular populations in PEC (%)				
	Total cells	Mϕ	B cells	Mϕ	B cells	T cells	Neutrophils	DCs
Y	20.8 ± 8.3	20 ± 0.4	0.6 ± 0.4	99 ± 2	3 ± 1	0.2 ± 0.1	0.2 ± 0.1	2.6 ± 0.8
O	38.8 ± 6.4*	36 ± 2*	4 ± 0.8*	95 ± 5	11 ± 2*	0.2 ± 0.2	0.5 ± 0.4	1.5 ± 0.3*

* p<0.01 compared to the young counterpart.

peritoneum from young mice, with an important reduction in the percentage of MØ ($CD11b^{high}$, $F4/80^{high}$). However, the absolute number of MØ was similar in old and young mice. Concomitantly, although a much higher percentage and a greater absolute number of B ($CD19^{+}$) cells were observed in the peritoneum of old mice (Table 1), the distribution of B cell subpopulations was similar in old and young mice (data not shown). When r-PC were cultured overnight without stimuli, IL-10 was the main cytokine secreted, with no significant differences in IL-10 or IL-12 secretion between ages (IL-10 (pg/ml): 238 ± 171 in old vs 375 ± 153 in young mice; IL-12 (pg/ml): 44 ± 14 in old vs 75 ± 32 in young mice). In addition, no difference was found in the basal arginase activity of MØ, without any basal production of NO being detectable in either group (data not shown).

Next, we examined whether aging changes the expression of molecules recognized as part of a pattern of MØ activation. The expression of macrophage mannose receptor (MMR), scavenger receptor type A (SRA) and CD23 on MØ as markers of alternative activation, as well as CD16/32 as a marker of classical activation, and MHC-II, CD80 and CD86 as general markers of MØ activation, was studied. First, we studied the expression of these molecules in MØ of r-PC. We did not found age-dependent bias in the expression of these molecules. What is more, expression of the alternative activation marker SRA was lower in MØ from aged mice. Also, MØ from old mice had a higher CD11b and lower F4/80 expression (Fig. 1A). This is in agreement with previous reports showing a higher expression of CD11b on splenic MØ from aged mice (Chelvarajan et al., 2005). Increased CD11b expression has also been observed after some pathological conditions (Kuo et al., 1996) and after *in vitro* LPS stimulation (Burges et al., 1997). The up-regulation of F4/80 expression from monocytes to differentiated tissue MØ has been associated to a site-specific anchoring function (Stacey et al., 2000) and consequently, the increased CD11b and reduced F4/80 expression in MØ from old mice could be associated with an enhanced ability of peritoneal MØ to migrate to the lymphoid organs, a critical process in the development of adaptive immunity. We did not find any difference in the expression of other molecules when comparing resident peritoneal MØ from young and old mice.

We continued this study by analyzing the behavior of MØ from old mice under an *in vivo* inflammatory stimulus. Overall, the reaction in the peritoneal cavity following thioglycollate injection was similar in both old and young mice, with an approximately 4-fold increase in the total number of PEC compared to the number of r-PC. As observed for r-PC, old mice had more PEC than young mice ($PEC \times 10^6$: 38.8 ± 6.4 in old vs 20.8 ± 8.3 in young mice, $p \leq 0.0001$). At both ages, MØ was the main cell population present in the exudates, forming more than 90% of the total cells (Table 1). Thus, probably the monocytes from old mice reach the inflammatory site and may also be capable of differentiating into MØ as in the case of monocytes from young mice. These observations suggest that monocytes from aged animals may conserve the ability to respond to tissue chemotactic stimuli. Thioglycollate-elicited inflammatory MØ exhibited a more activated phenotype than their resident counterparts, with an up-regulated expression of CD80 and CD86. The pattern of expression of these molecules was quite similar in MØ from young and old mice, showing that under inflammatory conditions, MØ from old mice could be ready to respond to injurious agents (Fig. 1B).

Taken together, these results support the idea that MØ obtained from the peritoneum of aged mice, in native or inflammatory conditions, do not show any of the commonly accepted markers of classical or alternative activation, and this could be of great importance in maintaining immune homeostasis. This is in agreement with a previous report demonstrating that spleen MØ from aged mice did not manifest an alternative activation phenotype (Chelvarajan et al., 2006).

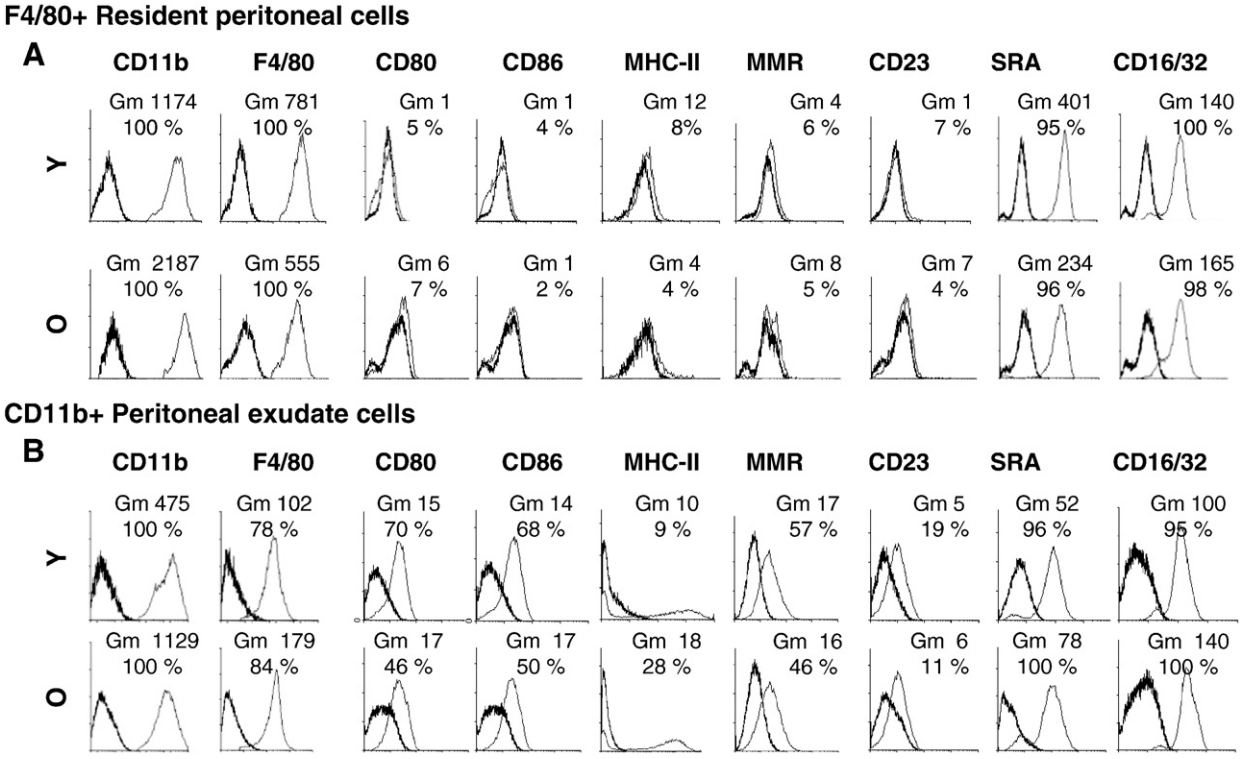


Fig. 1. Characterization of peritoneal macrophages of old mice. FACS analysis was performed for (A) r-PC (B) PEC obtained from young and old mice. Histograms indicate the surface antigen expression (thin line) compared with its respective isotype control (thick line). Data represent the geometric means of the intensity of fluorescence (Gm) of cells incubated with antibody minus the isotype control and the percentage of positive cells in relation to the total (A) F4/80+ cells or (B) CD11b + cells.

We have previously demonstrated that CpG-ODN was able to induce a specific Th1 response in aged mice comparable to that in young mice (Alignani et al., 2005; Maletto et al., 2002; Maletto et al., 2005). In order to extend these results, we studied the effect of CpG-ODN on MØ within their natural milieu. r-PC from young and old mice were cultured with CpG-ODN + IFN-γ, with IFN-γ being included because we had previously observed that certain MØ responses to CpG-ODN occurred only in the presence of this cytokine (Liscovsky et al., 2009).

CpG-ODN + IFN-γ induced IL-12 secretion in r-PC from young and from aged mice, but r-PC from old mice produce significantly less IL-12 but more IL-10 than their young counterparts, with a clear predominance of IL-10 over IL-12 production of r-PC in aged mice (Fig. 2A).

Our findings are consistent with previous reports from Sen et al. who observed that immunization of aged mice with a T cell-dependent antigen combined with CpG-ODN restored specific IgG responses to young adult levels. Interestingly they also found that spleen and peritoneal MØ from aged mice secreted higher levels of IL-10 in response to CpG-ODN, but in contrast with our results, they observed that aged and young adult mice made comparable levels of cytokines as IL-12 (Sen et al., 2006). In addition, Sharma et al. observed that intratumoral injections of CpG-ODN completely rejected the tumor in both young and old mice. Furthermore, the injection of CpG-ODN induced a strong pro-inflammatory response, in which the levels of IL-12 and other inflammatory molecules were significantly higher in young and old tumors than in untreated animals, although the level of IL-12 in treated old mice was lower than in treated young mice. They observed no changes in the level of IL-10 between young and old mice (Sharma et al., 2008).

We also observed that spleen cells from aged mice *in vitro* stimulated with CpG-ODN produced similar levels of IL-12 to young spleen cells (Maletto et al., 2002). Thus, we and others showed that Mϕ from aged mice were able to respond to CpG-ODN stimulation by secreting IL-12 cytokine in a similar way to cells from young mice. However, the data presented in this work established that aged cells stimulated with CpG-ODN + IFN-γ produced less IL-12 than young cells. Previously, we observed that CpG-ODN *in vitro* induce arginase activity in young murine MØ, but this occurred only in the presence of IFN-γ, revealing a singular effect of the combination of CpG-ODN + IFN-γ, one of the major cytokines produced in response to CpG-ODN administration *in vivo*. Therefore, there is the possibility that CpG-ODN may simultaneously or sequentially elicit both a pro-inflammatory and an anti-inflammatory response, similar to our *in vitro* data, with a different kinetic between young and old mice. In this context, r-PC from old mice had comparable arginase activity to that of young mice (Fig. 2B), but a lower NO production (Fig. 2C) when they are incubated in the presence of CpG-ODN + IFN-γ. Considering that, as mentioned above, r-PC from old mice had a much lower MØ proportion than young r-PC and that MØ were the principal NO producer cells in the peritoneum, NO secretion was normalized to the actual number of MØ presented in each sample (determined in parallel by FACS). This procedure clearly showed that NO production per MØ under CpG-ODN + IFN-γ stimulation was higher in old mice than in young mice (Fig. 2D), despite the higher IL-10 secretion by r-PC under CpG-ODN + IFN-γ stimulation, while no difference was found in arginase activity in MØ from mice of both ages. Nevertheless, in the peritoneum of aged mice, despite the anti-inflammatory milieu occurring upon activation with CpG-ODN + IFN-γ, MØ became activated producing some inflammatory as well as anti-inflammatory mediators.

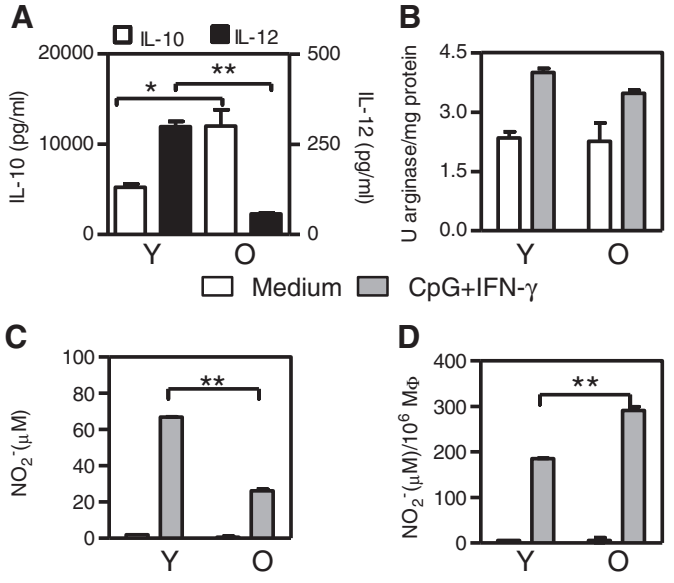


Fig. 2. Response of r-PC from old mice to CpG-ODN + IFN-γ. Equal numbers of r-PC from young and old mice were incubated with medium or with 3 μM CpG-ODN plus 50 UI/ml IFN-γ for 48 h. (A) IL-10 and IL-12 were determined in supernatants. Results represent the concentration of cytokines of the stimulated minus the unstimulated r-PC. (B) Arginase activity: r-PC cells were carefully washed several times to obtain a population of adherent cells and lysated to determine arginase activity. (C–D) NO_2^- concentration was determined in supernatants by the Griess reaction. The NO_2^- concentration is shown as the concentration obtained directly from the supernatants (C), and as the normalized NO_2^- concentration related to the number of MØ present in the sample (D). Results are representative of at least 3 experiments performed. (n: 3–4 mice per group). *, $p<0.05$; **, $p<0.01$.

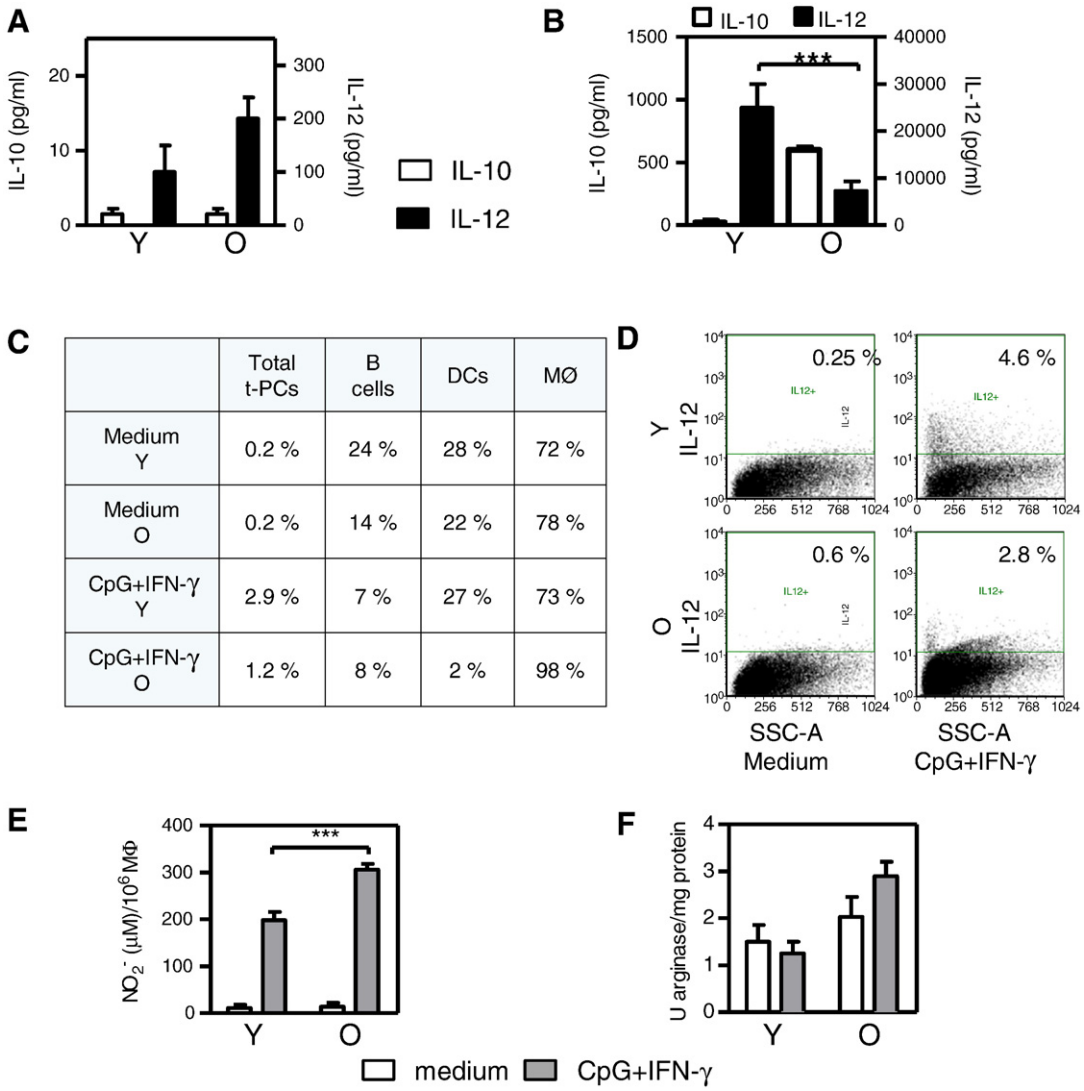
In order to investigate how activated MØ respond to a CpG stimulus, we evaluated the effect of CpG-ODN + IFN-γ on peritoneal cells after a thioglycollate i.p. injection. Incubation of PEC from both young and old mice without any stimulus produced less IL-10 than r-PC without stimulus (Fig. 3A). When CpG-ODN + IFN-γ were added to PEC, IL-10 secretion was only stimulated in old PEC. However, IL-12 secretion was strongly stimulated in PEC in both ages, although at a lower rate in old mice (Fig. 3B). For this condition, IL-12 was the principal cytokine secreted, independent of age. We found that all the main cell populations in PEC were able to produce IL-12 at both ages. However, MØ from PEC was the principal cell type responsible for IL-12 production, particularly in old mice (Fig. 3C–D). Finally, even though PEC from aged mice were found to secrete significantly greater amounts of NO than their younger counterparts (Fig. 3E), they failed to induce any arginase activity (Fig. 3F).

4. Conclusions

Summing up, MØ from old mice did not show any dramatic impairment in their function, either in native or in inflammatory conditions. Therefore, under the correct stimulus, MØ from old mice were able to mount an inflammatory response. In other words, the immune depression found in advanced age is probably not directly related to an intrinsic decline in MØ function. Understanding how the MØ fluctuate between inflammatory and anti-inflammatory mediators during aging is a key point for being able to manipulate immune responses and to propose subsequent therapeutic strategies. Our results suggest that efforts to enhance vaccine responses in aged individuals should include agents that can efficiently activate the MØ function. In this context, the increasing recognition of the immunostimulatory features, the safety profile, and more recently the auto-regulatory properties of CpG-ODN (Liscovsky et al., 2009; Mellor et al., 2005; Wingender et al., 2006) render it a very attractive tool.

Acknowledgments

This work was supported by grants from CONICET (PIP # 5750 and 11220090100109), Agencia Nacional de Promoción Científica y Técnica (PICT # 25552 and # 48) and SECyT-UNC to M.C.P.P. M.C.P.P. and G.M.



	Total t-PCs	B cells	DCs	MØ
Medium Y	0.2 %	24 %	28 %	72 %
Medium O	0.2 %	14 %	22 %	78 %
CpG+IFN-γ Y	2.9 %	7 %	27 %	73 %
CpG+IFN-γ O	1.2 %	8 %	2 %	98 %

Fig. 3. Response of PEC from old mice to CpG-ODN + IFN-γ. Peritoneal cells were obtained by lavages of the peritoneum injected 5 days previously with 1 ml of 4% thioglycollate. Equal numbers of PEC from young and old mice were incubated with medium or 3 μM CpG-ODN plus 50 UI/ml IFN-γ for 48 h. (A and B) IL-10 and IL-12 were determined by ELISA in the supernatants of PEC cultured without stimulus (A) or incubated with CpG-ODN + IFN-γ (B). (C–D) Intracellular IL-12 staining of PEC cultivated with or without CpG-ODN + IFN-γ. Cells were recovered and labeled with anti CD11c (for dendritic cells), anti-CD19 (for B cells), anti-CD11b (for MØ after gating out $CD11c^+$cells) and with anti-IL-12p40/p70. The table in C) shows the percentages of $IL\text{-}12^+$cells in total PEC and the relative composition of B cells, dendritic cells and MØ in $IL\text{-}12^+$PEC expressed as percentage. Results in D) show a representative dot plot analysis of intracellular IL-12 for $CD11c^+$ $CD11b^+$ cells, where the percentage of $IL\text{-}12^+$ cells is indicated. (E) The NO_2^- concentration was determined by Griess reaction in supernatants of PEC incubated with or without CpG-ODN + IFN-γ and is shown as NO_2^- concentration per 1×10^6 MØ. (F) Arginase activity was determined in lysates of adherent PEC incubated with or without CpG-ODN + IFN-γ. Results are shown as the mean ± SD of a representative experiment out of 3 performed. (n: 4–5 mice per group). ***, $p<0.001$.

are career members of CONICET. M.V.L; R.P.R and C.V.G are recipients of post-graduate fellowships from CONICET. We thank native speaker, Joss Heywood, who revised the manuscript.

References

Alignani, D., Maletto, B., Liscovsky, M., Ropolo, A., Moron, G., Pistoresi-Palencia, M.C., 2005. Orally administered OVA/CpG-ODN induces specific mucosal and systemic immune response in young and aged mice. J. Leukoc. Biol. 77, 898–905.

Burges, A., Allmeling, A., Krombach, F., 1997. Hyperoxia induces upregulation of CD11b and amplifies LPS-induced TNF-alpha release by alveolar macrophages. Eur. J. Med. Res. 2, 149–154.

Chelvarajan, R.L., Collins, S.M., Van Willigen, J.M., Bondada, S., 2005. The unresponsiveness of aged mice to polysaccharide antigens is a result of a defect in macrophage function. J. Leukoc. Biol. 77, 503–512.

Chelvarajan, R.L., Liu, Y., Popa, D., Getchell, M.L., Getchell, T.V., Stromberg, A.J., Bondada, S., 2006. Molecular basis of age-associated cytokine dysregulation in LPS-stimulated macrophages. J. Leukoc. Biol. 79, 1314–1327.

Corraliza, I.M., Campo, M.L., Soler, G., Modolell, M., 1994. Determination of arginase activity in macrophages: a micromethod. J. Immunol. Meth. 174, 231–235.

Corsini, E., Di Paola, R., Viviani, B., Genovese, T., Mazzon, E., Lucchi, L., Marinovich, M., Galli, C.L., Cuzzocrea, S., 2005. Increased carrageenan-induced acute lung inflammation in old rats. Immunology 115, 253–261.

De La Fuente, M., 1985. Changes in the macrophage function with aging. Comp. Biochem. Physiol. A Comp. Physiol. 81, 935–938.

Fietta, A., Merlini, C., De Bernardi, P.M., Gandola, L., Piccioni, P.D., Grassi, C., 1993. Non specific immunity in aged healthy subjects and in patients with chronic bronchitis. Aging Milano 5, 357–361.

Goerdt, S., Orfanos, C.E., 1999. Other functions, other genes: alternative activation of antigen-presenting cells. Immunity 10, 137–142.

Gordon, S., 2003. Alternative activation of macrophages. Nat. Rev. Immunol. 3, 23–35.

Hilmer, S.N., Cogger, V.C., Le Couteur, D.G., 2007. Basal activity of Kupffer cells increases with old age. J. Gerontol. A Biol. Sci. Med. Sci. 62, 973–978.

Kohut, M.L., Senchina, D.S., Madden, K.S., Martin, A.E., Felten, D.L., Moynihan, J.A., 2004. Age effects on macrophage function vary by tissue site, nature of stimulant, and exercise behavior. Exp. Gerontol. 39, 1347–1360.

Kuo, H.P., Ho, T.C., Wang, C.H., Yu, C.T., Lin, H.C., 1996. Increased production of hydrogen peroxide and expression of CD11b/CD18 on alveolar macrophages in patients with active pulmonary tuberculosis. Tuber. Lung Dis. 77, 468–475.

Liscovsky, M.V., Ranocchia, R.P., Gorlino, C.V., Alignani, D.O., Moron, G., Maletto, B.A., Pistoresi-Palencia, M.C., 2009. Interferon-gamma priming is involved in the activation of arginase by oligodeoxinucleotides containing CpG motifs in murine macrophages. Immunology 128, e159–e169.

Maletto, B., Ropolo, A., Moron, V., Pistoresi-Palencia, M.C., 2002. CpG-DNA stimulates cellular and humoral immunity and promotes Th1 differentiation in aged BALB/c mice. J. Leukoc. Biol. 72, 447–454.

Maletto, B.A., Ropolo, A.S., Liscovsky, M.V., Alignani, D.O., Glocker, M., Pistoresi-Palencia, M.C., 2005. CpG oligodeoxinucleotides functions as an effective adjuvant in aged BALB/c mice. Clin. Immunol. 117, 251–261.

Mellor, A.L., Baban, B., Chandler, P.R., Manlapat, A., Kahler, D.J., Munn, D.H., 2005. Cutting edge: CpG oligonucleotides induce splenic CD19+ dendritic cells to acquire potent indoleamine 2, 3-dioxygenase-dependent T cell regulatory functions via IFN Type 1 signaling. J. Immunol. 175, 5601–5605.

Miller, A.P., Xing, D., Feng, W., Fintel, M., Chen, Y.F., Oparil, S., 2007. Aged rats lose vasoprotective and anti-inflammatory actions of estrogen in injured arteries. Menopause 14, 251–260.

Mills, C.D., Kincaid, K., Alt, J.M., Heilman, M.J., Hill, A.M., 2000. M-1/M-2 macrophages and the Th1/Th2 paradigm. J. Immunol. 164, 6166–6173.

Mosser, D.M., 2003. The many faces of macrophage activation. J. Leukoc. Biol. 73, 209–212.

Sen, G., Chen, Q., Snapper, C.M., 2006. Immunization of aged mice with a pneumococcal conjugate vaccine combined with an unmethylated CpG-containing oligodeoxynucleotide restores defective immunoglobulin G antipolysaccharide responses and specific CD4+-T-cell priming to young adult levels. Infect. Immun. 74, 2177–2186.

Sharma, S., Dominguez, A.L., Hoelzinger, D.B., Lustgarten, J., 2008. CpG-ODN but not other TLR-ligands restore the antitumor responses in old mice: the implications for vaccinations in the aged. Cancer Immunol. Immunother. 57, 549–561.

Shearer, G.M., 1997. Th1/Th2 changes in aging. Mech. Ageing Dev. 94, 1–5.

Stacey, M., Lin, H.H., Gordon, S., McKnight, A.J., 2000. LNB-TM7, a group of seven-transmembrane proteins related to family-B G-protein-coupled receptors. Trends Biochem. Sci. 25, 284–289.

Stout, R.D., Suttles, J., 2004. Functional plasticity of macrophages: reversible adaptation to changing microenvironments. J. Leukoc. Biol. 76, 509–513.

Stout, R.D., Suttles, J., 2005. Immunosenescence and macrophage functional plasticity: dysregulation of macrophage function by age-associated microenvironmental changes. Immunol. Rev. 205, 60–71.

Wingender, G., Garbi, N., Schumak, B., Jungerkes, F., Endl, E., von Bubnoff, D., Steitz, J., Striegler, J., Moldenhauer, G., Tuting, T., Heit, A., Huster, K.M., Takikawa, O., Akira, S., Busch, D.H., Wagner, H., Hammerling, G.J., Knolle, P.A., Limmer, A., 2006. Systemic application of CpG-rich DNA suppresses adaptive T cell immunity via induction of IDO. Eur. J. Immunol. 36, 12–20.