



BIOMEDICAL SCIENCES

Lycopene induces bone marrow lymphopoiesis and differentiation of peritoneal IgA-producing cells

MONIQUE B. ELIAS, ANDERSON J. TEODORO, FELIPE S. LEMOS, EMERSON S. BERNARDES, SOFIA N. SANTOS, SIDNEY PACHECO & FELIPE L. DE OLIVEIRA

Abstract: Lycopene is a hydrocarbon-carotenoid commonly found in red fruits intake with major function correlated to antioxidative capacity in several pathological conditions, including cancer and cardiovascular diseases. Recently, lycopene has been associated with hematopoiesis, although the effects on B lymphocyte differentiation and antibody production are poorly understood. In this work, the principal aim was to investigate whether lycopene affects B lymphopoiesis and terminal differentiation into plasma cells. Distinct *in vivo* and *in vitro* strategies based on lycopene supplementation were used direct in Balb/c mice or in culture systems with cells derived of these mice. In the bone marrow, lycopene expanded B220⁺IgM⁻ progenitor B cells and B220⁺IgM⁻ immature B lymphocytes. In the spleen, lycopene induced terminal CD138⁺ plasma cell generation. In the blood, we found prominent IgA and low IgM levels after lycopene administration. Interestingly, the pattern of peritoneal IgM⁺ and IgA⁺ B cells indicated a significant IgM-to-IgA class switching after lycopene injection. These data indicated that lycopene induces B cell differentiation into IgA-producing plasma cells. Thus, a new cellular function has been attributed to lycopene for B lymphocyte biology and possibly associated with humoral responses and mucosal immunity.

Key words: B lymphocytes, cell differentiation, IgA, immunology, Lycopene.

INTRODUCTION

Lycopene is a hydrocarbon which belongs to carotenoid family found in red fruits and vegetables with antioxidant functions on cancer progression and cardiovascular diseases (Saini et al. 2020). The food consumption is predominantly based on tomato intake and cellular absorption of lycopene is essentially associated with maintenance of serum concentration. Its average consumption includes 5 to 7 mg/day in developed countries (Cruz Bojorquez et al. 2013). Although numerous biological effects have been described in distinct experimental and clinical conditions, the role of lycopene on B lymphocyte

differentiation and antibody production are poorly understood.

In the bone marrow, subpopulations of B lymphocytes are hallmarked by specific phenotypes, including Pre-Pro-B cells (B220^{low}CD19⁻IgM⁻ cells), Pro-B cells (B220^{low}CD19^{low}IgM⁻ cells), Pre-B cells (B220^{low}CD19^{low}IgM^{-/Low} cells) and Immature B cell (B220^{high}CD19^{high}IgM^{Low} cells). This last stage of bone marrow differentiation is characterized by cells that move to secondary lymphoid organs, including spleen and lymph nodes. Then, these cells proliferate (clonal expansion) and differentiate into CD138⁺ Immunoglobulin

(Ig)-secreting plasma cells (Eibel et al. 2014, Lim et al. 2017).

IgM is the first humoral line of body protection and normally represent 10% of total subtypes (Savage & Baumgarth 2015). On the other hand, IgA drives mucosal immune responses (Cerutti 2010). B lymphocytes expressing IgM (IgM⁺ B cells) can secrete IgG, IgE or IgA after appropriate Ig-class switching (Stavnezer & Schrader 2014). IgM-to-IgA class switching is hallmarked by CD138⁻B220⁺IgM⁺IgA⁻ B lymphocytes that differentiate into CD138⁺B220⁺IgM⁺IgA⁺ plasmablasts and, subsequently, CD138⁺B220⁻IgM⁻IgA⁺ plasma cells (Kunisawa & Kiyono 2012, Shikina et al. 2004). For the first time, the involvement of lycopene has been investigated at this context using *in vitro* and *in vivo* distinct strategies. Here, we found that lycopene induced B cell differentiation directly associated with prominent levels of IgA and low levels of IgM in the serum of lycopene-supplemented mice, indicating a new function by regulating IgM-to-IgA class switch during B lymphocyte differentiation.

MATERIALS AND METHODS

Mice

Male BALB/c mice aging 2 to 4 months were obtained from the Federal University of Rio de Janeiro (Brazil). The experimental protocols involving mice were in accordance with guidelines provided by Brazilian College of Animal Experimentation (CONCEA - Conselho Nacional de Controle de Experimentação Animal) and approved by the Animal Ethics Committee (CEUA, Comissão de Ética no Uso de Animais) of Federal University of Rio de Janeiro, Brazil (protocol number DAHEICB009). Five mice per experimental group were used in this work.

Bone marrow and splenic cell suspensions

Bone marrow cells were obtained by “flushing” in the femur ejecting the marrow content with needle and syringe with RPMI 1640, pH 7.4, 10% fetal bovine serum (LGC, Sao Paulo, Brazil). Spleen was removed after opened peritoneal cavity and splenocytes were isolated by *ex vivo* standard mechanical procedures and washed twice with PBS, pH 7.4, containing 3% FBS. Both cell suspensions were quantified by hemocytometer. Erythrocytes were lysed with hypotonic solution.

Lycopene supplementation

For *in vitro* studies, bone marrow and splenic cells were adjusted to 5×10^5 cells per 2 ml of RPMI 1640, pH 7.4, 10% FBS in 6-well plates at 37°C and 5% CO₂ atmosphere. Water-soluble all-trans lycopene (10%) was provided by Roche (Rio de Janeiro, Brazil), dissolved in water at 50°C and adjusted to 1uM, 5uM and 10uM. The culture medium was restored every 48h to maintain 2mL as final volume and concentration of lycopene. On day 5 of treatment, cells treated with lycopene were collected to analysis. Control groups were maintained in medium alone. For *in vivo* studies, lycopene 5μM (2.68 μg/mL) was daily administrated by intraperitoneal injection adjusted to 300 μL (final volume) continuously for 2 weeks. Control mice receipt distilled water (vehicle) at same final volume.

Flow cytometry – phenotypic analysis

Bone marrow and splenic cells were adjusted to 1×10^6 cells/mL. Before fluorescent-antibody staining, Fc receptors were blocked by Fc-IgG blocker (clone 2.4G2, Cell Bank of Rio de Janeiro, Brazil). Subsequently, cells were incubated with monoclonal antibodies for 30 minutes and unbound antibodies are washed with PBS. Monoclonal antibodies: anti-IgM FITC; anti-CD138 and anti-IgA PE; anti CD19 PE-Cy5.5;

anti-B220 APC (BD Bioscience, CA, USA). Samples were acquired and analyzed in FACScalibur flow cytometer (BD Bioscience) by Cell Quest Pro.

Flow cytometry – cell viability

Bone marrow cells (1×10^6 cells/mL) were treated with $5 \mu\text{M}$ and $10 \mu\text{M}$ of lycopene for 5 days and stained with Annexin-V FITC and propidium iodide (PI) following manufacturer instructions (BD Bioscience). Cytotoxicity was evaluated as follow: early apoptosis (Annexin-V⁺PI⁻) and late apoptosis (Annexin-V⁺PI⁺). Viable cells are double-negative (Annexin-V⁻PI⁻). Samples were acquired and analyzed in FACScalibur flow cytometer (BD Bioscience) using software Cell Quest Pro.

Optical microscopy

Cell suspensions were adjusted to 200ul from the 5.0×10^4 cells/mL solution to centrifugation at 36g for 3 minutes on glass slides (Thermo Scientific, USA). Samples were fixed in methanol and allowed to dry for 24 hours and stained by May-Grunwald & Giemsa method (el-Cheikh et al. 1991). Images were acquired using an Olympus CKX41 microscope with a Q-Color 3 (Olympus, USA) camera and Q-Capture software.

Immunocytochemistry

Splenic cell treated with lycopene (and respective control) were centrifuged onto glass slides coated with poly-L-lysine, fixed in methanol and allowed to dry for 24 hours at room temperature. After inhibition of endogenous peroxidase, cytosmears were incubated for 1h with 0.01M PBS containing 5% BSA, 4% skim milk, 0.1% Triton x-100 (Sigma Aldrich, USA), 0.05% Tween-20, and 10% normal goat serum and incubation with purified rat IgG anti-CD138 (BD Biosciences, USA), at 1:1800 in PBS 3% BSA for 4h at 4°C in a humid chamber. Antibodies were detected with a biotinylated anti-rat IgG (BA-4001, Vector

Laboratories, USA) and developed with avidin-peroxidase (1:100 in PBS) (Sigma Aldrich, USA), using diaminobenzidine (DAB) as chromogen diluted 1:50 in original diluent following manufacturer (Dako/Agilent, USA). Slides were counterstained with Harris'hematoxylin. Bright-field pictures were acquired using an Evolution MP 5.0 RTV Color camera (Media Cybernetics, Canada). As negative controls, specimens were incubated with non-immune rat serum instead of anti-CD138.

ELISA

The serum of mice supplemented with lycopene were collected on day 14 of treatment and analyzed individually by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Mabtech, Sweden). The optical density was measured by an ELISA microplate reader (Thermo Scientific, USA) and the absorbance values were directly proportional to the concentration of IgA and IgM in the serum.

Statistical analysis

Statistical data were performed using the Tukey's multiple comparison test (*t* test); $p \leq 0.05$ was considered statistically significant.

RESULTS

Lycopene supplementation induced B lymphopoiesis in vitro

Bone marrow cells were cultured in distinct concentrations of lycopene for 5 days. The number of hematopoietic cells was significantly increased when supplemented with lycopene $5 \mu\text{M}$ in comparison with control cells. In contrast, cellularity was similar to control condition when these cells were cultured with $1 \mu\text{M}$ and $10 \mu\text{M}$ of lycopene (Figure 1a). Phenotypic analysis

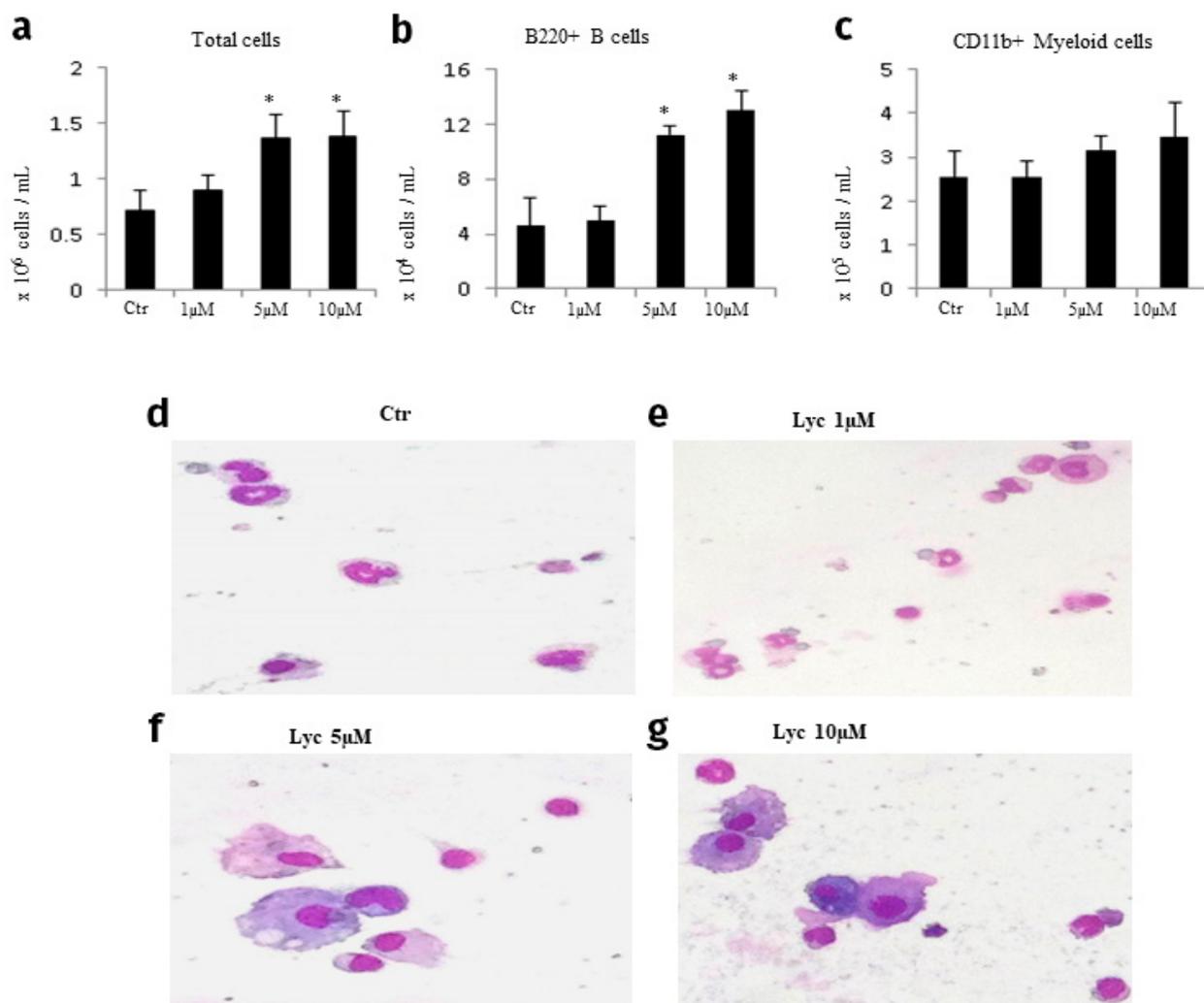


Figure 1. Definition of lycopene concentration to B cell differentiation analysis. (a) Cellularity of bone marrow cell culture. Number of B220⁺ B cells (b) and CD11b⁺ cells (c) after lycopene supplementation. Morphological analysis of cultured cells at distinct conditions: (d) control, medium alone; (e) lycopene 1µM; (f) Lycopene 5µM and (g) Lycopene 10µM. Magnification: 1000x. n = 5 mice/group. (*) Indicates p < 0.05.

revealed that lymphoid compartment was significantly modified by lycopene. On day 5, B220⁺ B cells were significantly increased after treatment with 5µM and 10µM of lycopene (Figure 1b). On the other hand, CD11b⁺B220⁻ myeloid cells were not modified in number after lycopene supplementation (Figure 1c). Morphological analysis revealed that polymorphonuclear cells were predominantly observed in control samples and 1µM of lycopene (Figure 1d and 1e, respectively). In contrast, mononuclear cells

were majorly found in 5µM and 10µM lycopene conditions (Figure 1f and 1g, respectively).

The cytotoxicity properties of lycopene on bone marrow cells were measured by flow cytometry. In control group, Annexin-V⁺PI⁻ early apoptotic cells and Annexin-V⁺PI⁺ late apoptotic cells represented 11.8% and 14.1% of cultured cells, respectively (Figure 2a). No significant differences were observed when bone marrow cells were supplemented with 5µM of lycopene (Figure 2b). On the other hand, lycopene 10µM

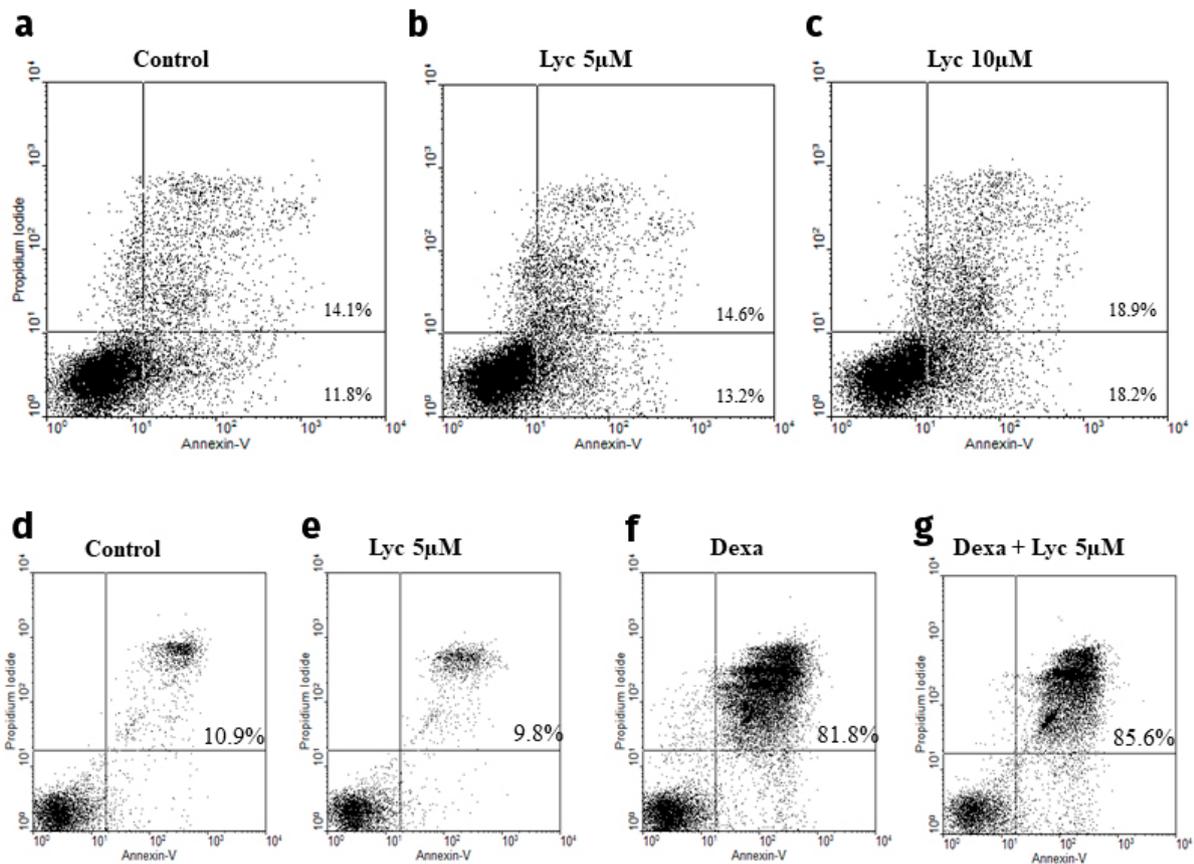


Figure 2. Bone marrow cell viability mediated by lycopene. Annexin-V and propidium iodide were used to measure cell viability in the bone marrow culture at control condition (a) and lycopene supplementation 5µM (b) and 10µM (c). Dexamethasone-induced apoptosis was used to evaluate possible anti-apoptotic effects of lycopene. (d) Control, medium alone; (e) Lycopene alone; (f) Dexamethasone alone; (g) Dexamethasone plus lycopene. Double-negative are viable cells; Annexin-V⁻PI⁻ are early apoptotic cells; Annexin-V⁺PI⁺ cells are late apoptotic cells; and Annexin-V⁺PI⁻ are non-apoptotic dead cells. n = 5 mice/group.

increased the percentage of Annexin-V⁻PI⁻ early apoptotic cells to 18.2% and Annexin-V⁺PI⁺ late apoptotic cells to 18.9% of total cells (Figure 2c). At this time, the results indicated that lycopene 1µM showed effects similar to control whereas lycopene 10µM induced more apoptosis in bone marrow cells than lycopene 5µM condition.

The concentration of lycopene 5µM induced significant responses on bone marrow B cell compartments. Moreover, at this concentration, the percentage of viable cells was compatible with control group. To study a possible regulatory role on pro-apoptotic signals, we

induced bone marrow cells to apoptosis using dexamethasone. On day 2 of culture, it was observed that lycopene 5µM did not interfere with dexamethasone-induced apoptosis (Figure 2d-2g). These data suggested that lycopene 5µM was able to induce B cell differentiation displaying very low cytotoxicity and poor interference with apoptotic signals, both events very important to B cell physiology.

Lycopene induces bone marrow B lymphocyte differentiation

B lymphocytes were minutely investigated after lycopene treatment *in vitro*. On day 5 of culture, the percentage of CD19⁺SSC^{low} total B cells was 13% of total cells. Phenotypic analysis of B cell subpopulations showed that CD19⁺B220^{low}IgM⁻ cells represented 18.9% of them whereas CD19⁺B220^{high}IgM⁻ and B220^{high}IgM^{high} cells were 51.5% and 29.1% of B cells, respectively, when cultured in medium alone (Figure 3a). Lycopene 5μM supplementation increased the percentage of B220⁺SSC^{low} total B cells (21% of total cells). The subpopulations represented 30.8%

(CD19⁺B220^{low}IgM⁻ cells), 37.7% (CD19⁺B220^{high}IgM⁻ cells) and 30% (CD19⁺B220^{high}IgM^{high} cells) of B cells in culture (Figure 3b).

The absolute number of these cell subtypes was defined according to total cellularity described in the figure 1. Lycopene 5μM induced significant changes in all B cell subpopulations: CD19⁺IgM⁻ B cells (1 to 3 x 10⁴ cells, Figure 3c), CD19⁺IgM⁻ B cells (2 to 4.5 x 10⁴ cells, Figure 3d), and CD19⁺IgM⁺ B cells (1.5 to 4 x 10⁴ cells, Figure 3E). *In vivo* lycopene supplementation showed very similar results. Mice intraperitoneally supplemented with 5μM of lycopene had significant increase of CD19⁺IgM⁻ progenitor

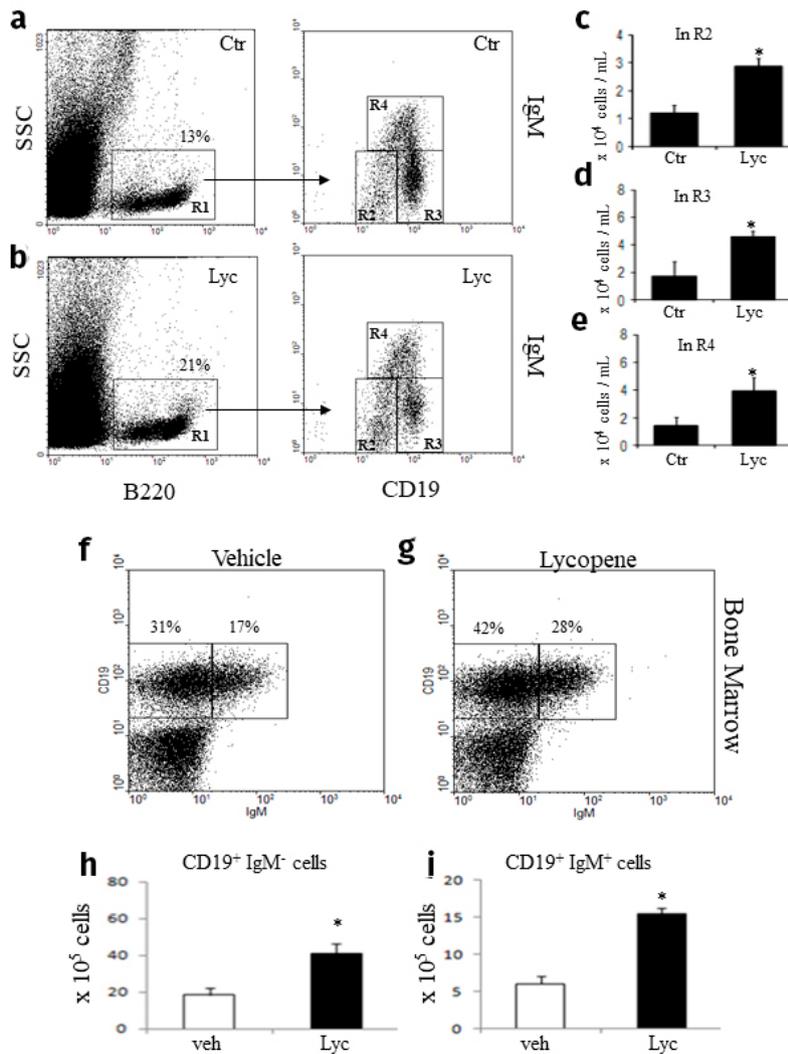


Figure 3. Phenotype of bone marrow B cells cultured with lycopene. B220⁺SSC^{low} B lymphocytes were gated and analyzed following CD19 and IgM expression in control (a, gate R1) and lycopene-supplemented group (b, gate R1). B220⁺CD19⁺IgM⁻ Pre-Pro B cells (c, gate R2), B220⁺CD19⁺IgM⁻ Pre B cells (d, gate R3), and B220⁺CD19⁺IgM⁺ Immature B cells (e, gate R4) were quantified after lycopene 5μM supplementation *in vitro*. In mice, the phenotype of bone marrow B lymphocytes was defined according the expression CD19 and IgM in control group (f) and 14 days after daily lycopene supplementation (g). (h) CD19⁺IgM⁻ B cells and (i) CD19⁺IgM⁺ B cells were significantly increased after lycopene treatment *in vivo*. n = 5 mice per experiment. * indicates p < 0.05.

B cells and CD19⁺IgM⁺ immature B cells, when compared with control (vehicle) mice (Figure 3f-3i).

Lycopene interferes with B cell differentiation in the spleen

Splenocytes were also isolated and cultured at lycopene 5 μ M condition. On day 5 of culture, lycopene induced a 3-fold increase in the number of CD138⁺ cells (a mix of plasmablasts and plasma cells). The number of these cells was approximately 0.5 $\times 10^4$ in control group (medium alone) and about 1.5 $\times 10^4$ cells after

lycopene supplementation (Figure 4a). These cells were identified by CD138 expression, large-oval cytoplasm, and eccentric nucleus (Figure 4b).

The intraperitoneal supplementation also disturbed splenic differentiation of B lymphocytes into plasma cells. On day 15 of lycopene-treatment, the percentage of splenic CD138⁺ plasma cells was significantly increased when compared with control mice (Figure 4c-4f). Plasmablasts (CD138⁺B220⁺ cells) were equivalent to 3% of splenic cells in control mice while this percentage changed to 8% in

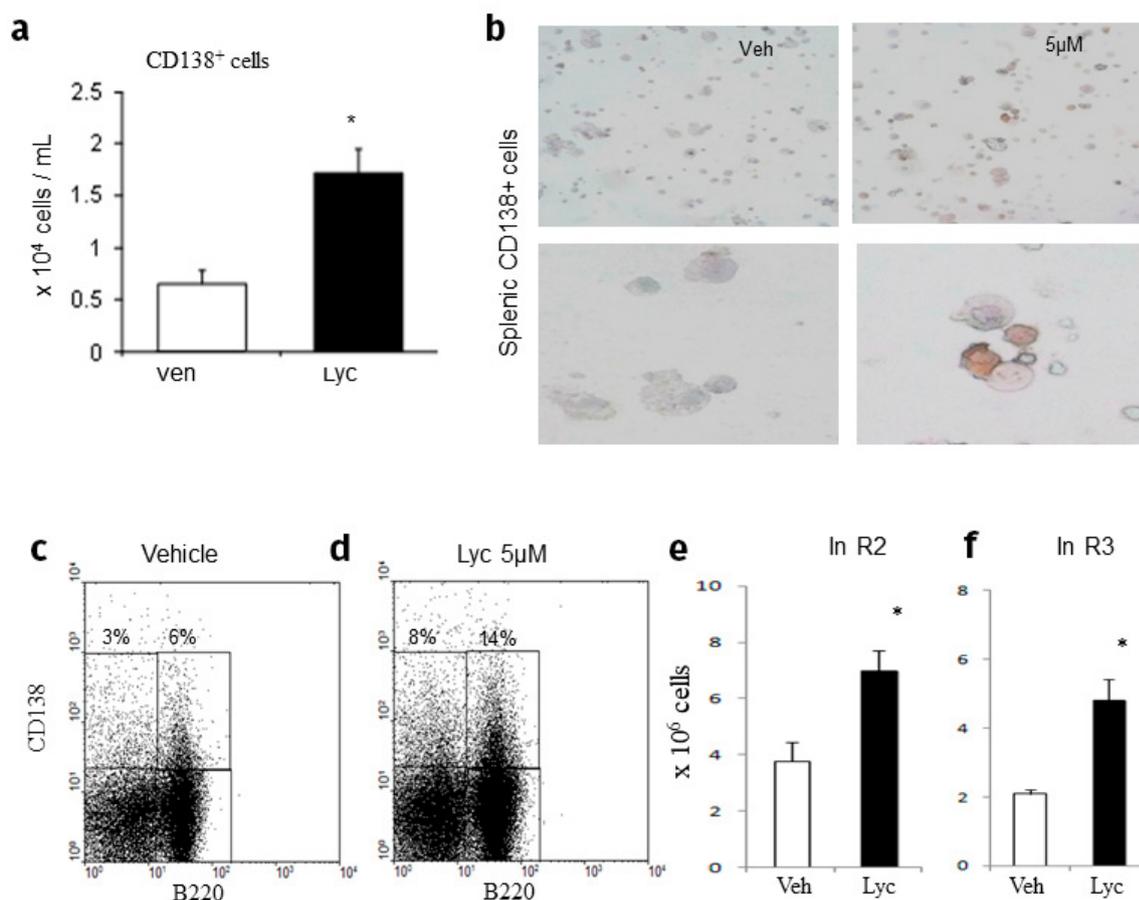


Figure 4. Splenic B cell differentiation induced by lycopene. Splenic cells cultured in the absence (a, control) and in contact with lycopene 5 μ M (a, Lyc) were quantified after 5 days of culture. (b) Immunocytochemistry essay revealed CD138⁺ cells numerically increased after lycopene supplementation *in vitro*. B220⁺CD138⁺ cells (plasmablasts) and B220⁻CD138⁺ cells (plasma cells) were quantified in control (c) and lycopene supplementation condition (d). The absolute number was defined according previous phenotype: Plasmablasts (e) and plasma cells (f). n=5 mice per group. * indicates p<0,05.

lycopene-supplemented mice (Figure 4c and 4d). In parallel, plasma cells (CD138⁺B220⁻ cells) represented 6% in control mice and 14% in lycopene-supplemented mice (Figure 4c and 4d). The absolute number corroborated with these data. In control mice, CD138⁺B220⁻ plasmablasts and CD138⁺B220⁻ plasma cells corresponded to approximately 4 x 10⁶ and 2 x 10⁶ splenocytes, respectively. On day 15 of *in vivo* lycopene-supplementation, these values increased to approximately 7.5 x 10⁶ and 5 x 10⁶ splenocytes, respectively (Figure 4e and 4f). These data suggested that lycopene induced B cell differentiation at distinct levels and organs.

An important functional parameter to measure the efficiency of B cell differentiation induced by lycopene supplementation is the concentration of Ig levels in the serum of mice submitted to lycopene intervention. The serum levels of IgM and IgA were significantly changed on day 15 of 5µM lycopene-supplementation. These mice were marked by reduced levels of IgM and elevated levels of IgA (Figure 5a and 5b). However, intraperitoneal injection of lycopene was not able to induce IgA⁺ cells in the bone marrow and spleen (Figure 5c and 5d).

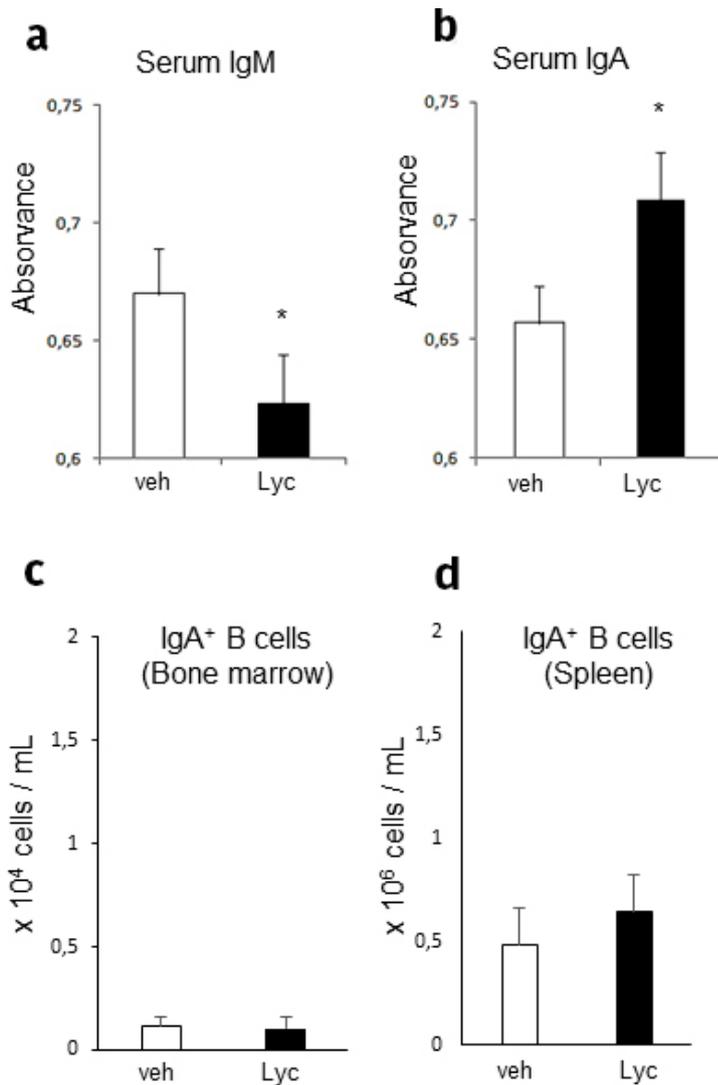


Figure 5. IgM and IgA levels modulated by lycopene. Serum levels of IgM (a) and IgA (b) were quantified by ELISA. The number of IgA⁺ B cells was measured in the bone marrow (c) and spleen (d). Veh indicates control mice treated with vehicle (water) while Lyc represents lycopene-supplemented mice. n=5 mice per group. * indicates p<0,05.

Lycopene favors Ig-class switching from IgM to IgA in the peritoneal cavity

The higher levels of IgA concomitant with indifferences observed in the number of splenic IgA⁺ cells led us to analyze peritoneal B compartments. Lycopene injection reduced the percentage of total peritoneal leukocytes (Figure 6a-6b). In particular, peritoneal B220⁺ B cells were numerically reduced on day 15 of lycopene administration (Figure 6c). Multiparametric analysis revealed a partial decrease of surface expression of IgM after lycopene treatment (Figure 6d). In parallel, these mice showed IgA⁺ B cells as result of lycopene supplementation (Figure 6e). The absolute numbers of peritoneal IgM⁺ B cells changed from 6 x10⁵ lymphocytes

in control mice to 3 x 10⁵ lymphocytes in mice supplemented with lycopene 5µM (Figure 6f). In contrast, peritoneal IgA⁺ B cells changed from 0.5 x10⁵ lymphocytes in control mice to approximately 2 x 10⁵ lymphocytes in mice after lycopene 5µM supplementation (Figure 6g). Together, these data indicated that lycopene affects peritoneal B cell compartments inducing IgM-to-IgA class switching.

DISCUSSION

For the first time, lycopene has been described as immunomodulatory molecule favoring IgM-to-IgA class switching and IgA⁺ B cell differentiation. Moreover, we have demonstrated that lycopene

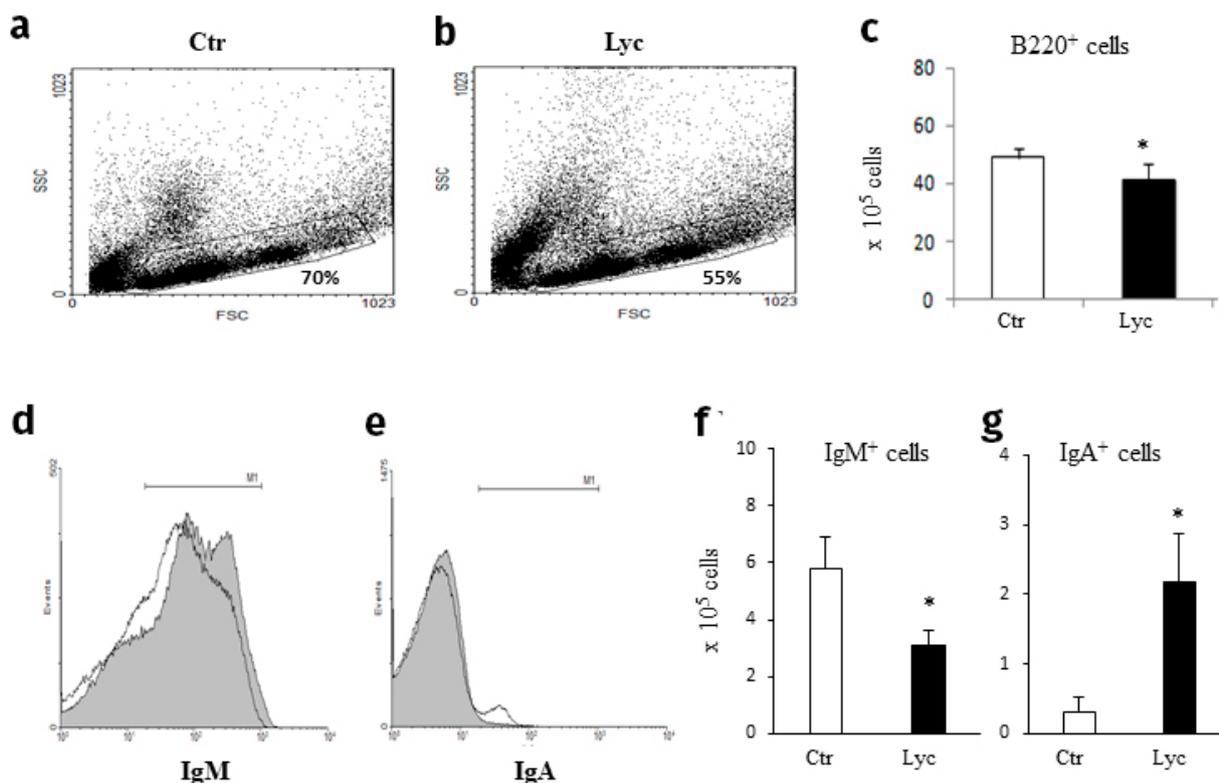


Figure 6. Effects of lycopene in the peritoneal B cell compartments. Cellular distribution of peritoneal floating cells harvested from control mice (a) and group intraperitoneally induced with lycopene 5µM (b). The number of total B220⁺ B cells was plotted in graph bars (c). The surface expression of IgM (d) and IgA (e) was plotted as overlaid histograms. Gray and white curves indicate control and lycopene-supplemented mice, respectively. The absolute number of IgM⁺ B cells (f) and IgA⁺ B cells (g) reveals possible IgM-to-IgA class switching on peritoneal B cells. n=5 mice per group. * indicates p<0,05.

induced bone marrow B lymphopoiesis and terminal differentiation into splenic plasma cells. Interestingly, serum levels of IgA were significantly increased on day 15 of lycopene supplementation. Our data can be included in protocols of experimental therapies based on improvement of IgA responses and kinetics of B cell recruitment in experimental models of diseases.

IgA has critical role to mucosal immune system controlling the gut microbiota to promote health (Macpherson et al. 2012). Among several reasons, IgA contributes to maintenance of gastrointestinal integrity when exposed to food antigens (Corthesy 2013). There are several authors indicating that IgA and gut barrier play an interesting crosstalk to control intestinal microbiota and gut-liver axis functions (Karaivazoglou et al. 2020, Wells et al. 2017). Moreover, these aspects have been associated with neurological disorders and mechanisms with potential to therapeutic strategies (Doenya 2019, Frasinariu et al. 2013, Mohamadkhani 2018). Our data suggested that supplementation using lycopene 5 μ M was optimal to drive these IgA-dependent biological conditions. Thus, we suggested that studies based on lycopene administration should be included in strategies to improve IgA responses.

Here, we used three different concentrations of lycopene (1, 5 and 10 μ M). Our data revealed that lycopene 1 μ M was similar to control in distinct essays. On the other hand, lycopene 10 μ M showed similarities with 5 μ M, however, the higher concentration had important cytotoxicity. Then, lycopene 5 μ M was considered ideal to continue this work, although it can be considered a supraphysiological condition. It was showed that 0.6 μ M of lycopene is the average in healthy human serum (Hoppe et al. 2003). In this work, we used an isomeric content of synthetic lycopene with equivalent bioavailability to

natural lycopene (Michael McClain & Bausch 2003). Previous results reinforced that lycopene 5 μ M was responsive to distinct experimental conditions, including cancer and normal cells (Haddad et al. 2016, Teodoro et al. 2012).

The consumption of 5-10mg/day of lycopene is sufficient to obtain benefits, but possibly, higher doses are required to affect immune cells (Arathi et al. 2016, Rao 2002, Rao & Agarwal 2000). In this work, we noted that 10 μ M of lycopene (5.36 μ g/mL) was significant cytotoxic to hematopoietic cells. It is plausible to suggest that 1 μ M of lycopene (0.536 μ g/Kg) is close to physiological concentration, while 5 μ M (2.68 μ g/mL) could be used as therapeutic strategy. There are several clinical situations hallmarked by IgA deficiencies extremely correlated with allergy, autoimmune responses, common variable immunodeficiency and common primary immunodeficiency (Wang & Hammarstrom 2012, Yazdani et al. 2016).

During immune responses, lycopene was described as repressor of IFN- γ and IL-4 synthesis by T lymphocytes, IL-2/IL-10 secretion by mononuclear blood cells *in vitro*, and airway inflammation in asthma model (Hazlewood et al. 2011, Kim et al. 2004, Yamaguchi et al. 2010). Nevertheless, the influence of lycopene on B cell activation and differentiation was poorly understood. Our results demonstrated that lycopene treatment *in vitro* and *in vivo* increased both IgM⁻ progenitors and IgM⁺ immature B lymphocytes derived from the bone marrow. In the spleen, lycopene supplementation was directly correlated with expansion of B220⁺CD138⁺ plasmablasts and B220⁺CD138⁺ plasma cells. In the peritoneal cavity, lycopene supplementation played positive role in IgA⁺ B cell differentiation. Together, these data pointed to stimulatory effects of lycopene to B cell lineage in distinct lymphoid tissues.

Low levels of IgM concomitant with high concentration of IgA in the serum indicated that lycopene administration was favoring IgM-to-IgA class switching (Cerutti & Rescigno 2008, Puga et al. 2010). Although no differences were found in the spleen regarding to IgA⁺ cells, the number of these cells was significantly increased in the peritoneal compartments. The peritoneal cavity is a critical B cell milieu responsible to maintain the mucosal immunity and IgA⁺ B cell repertoire, including conventional B2 and resident B1 lymphocytes (Hastings et al. 2006, Oliveira et al. 2015). However, it is important to note that IgM constitutes the first line of humoral defense against microbial and viral infections (Klimovich 2011).

Data showed here revealed a new function for lycopene as nutritional supplements, maybe, a nutraceutical compound to be used as immunomodulatory molecule to start or restore IgA-mediated immune responses. It was demonstrated that purified lycopene affected directly bone marrow B lymphopoiesis, splenic B cell differentiation into Ig-secreting plasma cells and IgM-to-IgA class switching in peritoneal B cells leading to IgA⁺ B cell generation. Data exposed in this work suggested that lycopene supplementation can be an excellent strategy to study immune responses associated with IgA mechanisms and experimental models of diseases characterized by cellular responses based on B lymphocytes.

Acknowledgments

We thank the Food and Nutrition Program (PPGAN) where M.B.E. performed Master and Doctoral degrees, and Morphological Sciences Program (PCM) where F.S.L. (CNPq scholarship) courses the Doctoral stage. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

REFERENCES

- ARATHI BP, SOWMYA PR, KURIAKOSE GC, VIJAY K, BASKARAN V, JAYABASKARAN C & LAKSHMINARAYANA R. 2016. Enhanced cytotoxic and apoptosis inducing activity of lycopene oxidation products in different cancer cell lines. *Food Chem Toxicol* 97: 265-276.
- CERUTTI A. 2010. Immunology. IgA changes the rules of memory. *Science* 328(5986): 1646-1647.
- CERUTTI A & RESCIGNO M. 2008. The biology of intestinal immunoglobulin A responses. *Immunity* 28(6): 740-750.
- CORTHESEY B. 2013. Role of secretory IgA in infection and maintenance of homeostasis. *Autoimmun Rev* 12(6): 661-655.
- CRUZ BOJORQUEZ RM, GONZALEZ GALLEGUO J & SANCHEZ COLLADO P. 2013. [Functional properties and health benefits of lycopene]. *Nutr Hosp* 28(1): 6-15.
- DOENYAS C. 2019. Novel Personalized Dietary Treatment for Autism Based on the Gut-Immune-Endocrine-Brain Axis. *Front Endocrinol (Lausanne)* 10: 508.
- EIBEL H, KRAUS H, SIC H, KIENZLER AK & RIZZI M. 2014. B cell biology: an overview. *Curr Allergy Asthma Rep* 14(5): 434.
- EL-CHEIKH MC, DUTRA HS & BOROJEVIC R. 1991. Eosinophil granulocyte proliferation and differentiation in schistosomal granulomas are controlled by two cytokines. *Lab Invest* 64(1): 93-97.
- FRASINARIU OE, CECCARELLI S, ALISI A, MORARU E & NOBILI V. 2013. Gut-liver axis and fibrosis in nonalcoholic fatty liver disease: an input for novel therapies. *Dig Liver Dis* 45(7): 543-551.
- HADDAD NF ET AL. 2016. Correction: Lycopene and Beta-Carotene Induce Growth Inhibition and Proapoptotic Effects on ACTH-Secreting Pituitary Adenoma Cells. *PLoS One* 11(2): e0149157.
- HASTINGS WD, TUMANG JR, BEHRENS TW & ROTHSTEIN TL. 2006. Peritoneal B-2 cells comprise a distinct B-2 cell population with B-1b-like characteristics. *Eur J Immunol* 36(5): 1114-1123.
- HAZLEWOOD LC, WOOD LG, HANSBRO PM & FOSTER PS. 2011. Dietary lycopene supplementation suppresses Th2 responses and lung eosinophilia in a mouse model of allergic asthma. *J Nutr Biochem* 22(1): 95-100.
- HOPPE PP, KRAMER K, VAN DEN BERG H, STEENGE G & VAN VLIET T. 2003. Synthetic and tomato-based lycopene have identical bioavailability in humans. *Eur J Nutr* 42(5): 272-278.

- KARAIVAZOGLU K, KONSTANTAKIS C, TOURKOCHRISTOU E, ASSIMAKOPOULOS SF & TRIANTOS C. 2020. Non-alcoholic fatty liver disease in inflammatory bowel disease patients. *Eur J Gastroenterol Hepatol* 32(8): 903-906.
- KIM GY, KIM JH, AHN SC, LEE HJ, MOON DO, LEE CM & PARK YM. 2004. Lycopene suppresses the lipopolysaccharide-induced phenotypic and functional maturation of murine dendritic cells through inhibition of mitogen-activated protein kinases and nuclear factor-kappaB. *Immunology* 113(2): 203-211.
- KLIMOVICH VB. 2011. IgM and its receptors: structural and functional aspects. *Biochemistry (Mosc)* 76(5): 534-549.
- KUNISAWA J & KIYONO H. 2012. Immunological function of sphingosine 1-phosphate in the intestine. *Nutrients* 4(3): 154-166.
- LIM VY, ZEHENTMEIER S, FISTONICH C & PEREIRA JP. 2017. A Chemoattractant-Guided Walk Through Lymphopoiesis: From Hematopoietic Stem Cells to Mature B Lymphocytes. *Adv Immunol* 134: 47-88.
- MACPHERSON AJ, GEUKING MB & MCCOY KD. 2012. Homeland security: IgA immunity at the frontiers of the body. *Trends Immunol* 33(4): 160-167.
- MICHAEL MCCLAIN R & BAUSCH J. 2003. Summary of safety studies conducted with synthetic lycopene. *Regul Toxicol Pharmacol* 37(2): 274-285.
- MOHAMADKHANI A. 2018. Gut Microbiota and Fecal Metabolome Perturbation in Children with Autism Spectrum Disorder. *Middle East J Dig Dis* 10(4): 205-212.
- OLIVEIRA FL, BERNARDES ES, BRAND C, DOS SANTOS SN, CABANEL MP, ARCANJO KD, BRITO JM, BOROJEVIC R, CHAMMAS R & EL-CHEIKH MC. 2015. Lack of galectin-3 up-regulates IgA expression by peritoneal B1 lymphocytes during B cell differentiation. *Cell Tissue Res* 363(2): 411-426.
- PUGA I, COLS M & CERUTTI A. 2010. Innate signals in mucosal immunoglobulin class switching. *J Allergy Clin Immunol* 126(5): 889-895; quiz 896-897.
- RAO AV. 2002. Lycopene, tomatoes, and the prevention of coronary heart disease. *Exp Biol Med (Maywood)* 227(10): 908-913.
- RAO AV & AGARWAL S. 2000. Role of antioxidant lycopene in cancer and heart disease. *J Am Coll Nutr* 19(5): 563-569.
- SAINI RK, RENGASAMY KRR, MAHOMOODALLY FM & KEUM YS. 2020. Protective effects of lycopene in cancer, cardiovascular, and neurodegenerative diseases: An update on epidemiological and mechanistic perspectives. *Pharmacol Res* 155: 104730.
- SAVAGE HP & BAUMGARTH N. 2015. Characteristics of natural antibody-secreting cells. *Ann N Y Acad Sci*.
- SHIKINA T, HIROI T, IWATANI K, JANG MH, FUKUYAMA S, TAMURA M, KUBO T, ISHIKAWA H & KIYONO H. 2004. IgA class switch occurs in the organized nasopharynx- and gut-associated lymphoid tissue, but not in the diffuse lamina propria of airways and gut. *J Immunol* 172(10): 6259-6264.
- STAVNEZER J & SCHRADER CE. 2014. IgH chain class switch recombination: mechanism and regulation. *J Immunol* 193(11): 5370-5378.
- TEODORO AJ, OLIVEIRA FL, MARTINS NB, MAIA GEA, MARTUCCI RB & BOROJEVIC R. 2012. Effect of lycopene on cell viability and cell cycle progression in human cancer cell lines. *Cancer Cell Int* 12(1): 36.
- WANG N & HAMMARSTROM L. 2012. IgA deficiency: what is new? *Curr Opin Allergy Clin Immunol* 12(6): 602-608.
- WELLS JM ET AL. 2017. Homeostasis of the gut barrier and potential biomarkers. *Am J Physiol Gastrointest Liver Physiol* 312(3): G171-G193.
- YAMAGUCHI M, HASEGAWA I, YAHAGI N, ISHIGAKI Y, TAKANO F & OHTA T. 2010. Carotenoids modulate cytokine production in Peyer's patch cells ex vivo. *J Agric Food Chem* 58(15): 8566-8572.
- YAZDANI R, AZIZI G, ABOLHASSANI H & AGHAMOHAMMADI A. 2016. Selective IgA deficiency: Epidemiology, Pathogenesis, Clinical Phenotype, Diagnosis, Prognosis and Management. *Scand J Immunol* 85(1): 3-12.

How to cite

ELIAS MB, TEODORO AJ, LEMOS FS, BERNARDES ES, SANTOS SN, PACHECO S & OLIVEIRA FL. 2022. Lycopene induces bone marrow lymphopoiesis and differentiation of peritoneal IgA-producing cells. *An Acad Bras Cienc* 94: e20210002. DOI 10.1590/0001-376520220210002.

*Manuscript received on January 2, 2021;
accepted for publication on February 10, 2022*

MONIQUE B. ELIAS¹

<https://orcid.org/0000-0001-5062-1401>

ANDERSON J. TEODORO¹

<https://orcid.org/0000-0002-0949-9528>

FELIPE S. LEMOS²

<https://orcid.org/0000-0003-3133-8373>

EMERSON S. BERNARDES³

<https://orcid.org/0000-0002-0029-7313>

SOFIA N. SANTOS³

<https://orcid.org/0000-0001-8795-316X>

SIDNEY PACHECO⁴

<https://orcid.org/0000-0002-4248-2365>

FELIPE LEITE DE OLIVEIRA²

<https://orcid.org/0000-0001-6193-1616>

¹Federal University of State of Rio de Janeiro (UNIRIO), Av. Pasteur, 296, Botafogo, 22290-250 Rio de Janeiro, RJ, Brazil

²Federal University of Rio de Janeiro, Institute of Biomedical Sciences, Avenida Carlos Chagas, 373, Ilha do Fundão 21941-902 Rio de Janeiro, RJ, Brazil

³Institute of Energy and Nuclear Research (IPEN), Radiopharmacy Center, Av. Prof. Lineu Prestes, 2242, Butantã 05508-000 São Paulo, SP, Brazil

⁴Embrapa Food Technology, Av. das Américas, 29501, Guaratiba, 23020-470 Rio de Janeiro, RJ, Brazil

Correspondence to: **Felipe Leite de Oliveira**

E-mail: felipe@histo.ufrj.br

Author contributions

M.B.E., A.J.T., and F.L.O. designed the study and experiments. M.B.E., F.S.L., E.S.B., S.N.S., S.P., A.J.T., and F.L.O. performed experiments, analyzed and discussed data, and prepared the text. All authors participated writing the manuscript.

