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Full Length Research Paper

Sensitization with babassu mesocarp induces activation of murine splenocytes against tumor cells

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***Attallea speciosa* Mart. (babassu) fruit contains a mesocarp that is rich in carbohydrates with immunomodulatory effects. The induction of the tolerogenic response is a tumor escape mechanism, and immunomodulator adjuvants have been studied to reestablish host immunogenicity. This study evaluates the adjuvant potential of babassu mesocarp carbohydrates in a tumor model. The babassu mesocarp extraction (BME) yield was 75.54%, and the total sugar concentration was 29.79 mg ml^{-1} containing monosaccharides, reducing sugars, polysaccharides and 0.506 mg ml^{-1} total protein. Chromatography analysis identified glucose, sucrose and fructose. Sensitization increased the spleen weight in the tumor group compared with the control, and a comparatively lower frequency of T helper and higher frequency of B-lymphocytes was also observed. The tumor+BME group had more cytotoxic T lymphocytes compared with the control. After co-culture with cancer cells, the tumor splenocytes showed lower proliferation, lower frequency of T helper cells and higher concentrations of interleukin (IL)-2, IL-6 and IL-10. However, the tumor+BME splenocytes presented results similar to the control, suggesting a reduction in the regulatory response of the tumor group. These results demonstrated that BME sensitization with cancer cells modulated an immune response in Balb/c animals, indicating an immunogenic effect.**

Key words: Arecaceae, *Attallea speciosa* Mart., adjuvant, carbohydrate, antitumor.

INTRODUCTION

The palm tree *Attallea speciosa* Mart., known as babassu, is a common species in Northeastern Brazil. The mesocarp of babassu fruit predominantly consists of carbohydrates (Silva and Parente, 2001) and is popularly used as a health food. Previous studies have demonstrated that the babassu mesocarp has been

traditionally used to treat inflammatory diseases (Agra et al., 2007), and the native communities have reported the use of babassu mesocarp to treat gastritis, leukorrhea and rounds (Souza et al., 2011). The biological effects of babassu mesocarp have been reported, including anti-inflammatory effects (Silva and Parente, 2001; Baldez, 2006; Nascimento et al., 2010; Silva et al., 2015). However, previous studies have shown that aqueous babassu mesocarp extract induces both *in vitro* and *in vivo* nitric oxide (NO) and tumor necrosis factor α (TNF-α) production in peritoneal macrophages (Nascimento et al., 2006). In addition, immunomodulatory (Guerra et al., 2011; Pessoa et al., 2014) and antitumoral effects (Rennó et al., 2008; de Souza et al., 2011; de Sousa et al., 2013) have been reported.

Herbal immunoadjuvants are substances that stimulate the immune response (Khyati, 2012), and carbohydrates are promising adjuvants because of their low cytotoxicity, good biocompatibility, and strong immune enhancement (Li and Wang, 2015).

Immune responses against tumors involve both innate and adaptive immunity (Lehrnbechter et al., 2008; Loose and Van de Wiele, 2009; Achyut and Arbab, 2016). Innate immunity includes natural killer (NK) cells and macrophages, while adaptive immunity involves cytotoxic T lymphocyte (CTL) cells, which are responsible for a more specific response against tumor antigens and the establishment of an immunologic memory (Klebanoff et al., 2006; Coulie et al., 2014; Dimeloe et al., 2016). Therefore, drugs with broad-spectrum activities, combining antitumoral and immunomodulatory effects with the capacity to mobilize the host immune system towards immunogenicity, are of great interest.

Considering its immunomodulatory activity and composition that is rich in carbohydrates, we hypothesized that the carbohydrates extracted from babassu mesocarp could act as adjuvants in sensitization against tumor cells. This study shows the *ex vivo* phenotypic, functional and cytokine production characteristics of splenocytes obtained from Balb/c mice sensitization using the extract of babassu mesocarp and tumor cells.

MATERIALS AND METHODS

Extract preparation and carbohydrate analysis

To prepare the babassu mesocarp extract (BME), the powder of babassu mesocarp supplied by the Cooperative of Coconuts breakers of Maranhão, located in Esperantinópolis-MA (Latitude: 04° 52' 00" S, Longitude: 44 42' 30" W) was used. The powder showed similarity regarding all of the botanical and phytochemical aspects compared with the mesocarp flour prepared in the laboratory (Nascimento et al., 2006). The botanical identifications were obtained from the Herbario Ático Seabra, State of Maranhão, Brazil (authenticated voucher specimen number 1135).

The babassu mesocarp powder was macerated in water at a concentration of 20 mg/ml for 24 h (Fortes et al., 2009), and subsequently, an aqueous babassu mesocarp extract was obtained (BME). The quantitative carbohydrate analysis was performed using a phenol-sulfuric acid method, with glucose as a standard (Dubois et al., 1956). The extract sample was deionized with a cationic-exchange column (Dowex 50W X 8 - 100) and anionic-exchange column (Dowex 1 X 8 -100). Next, the sample was filtered through 0.45-µm membrane filters. Samples with equivalent glucose concentrations (400 ug ml^{-1}) were analyzed by anion exchange chromatography coupled with pulsed amperometric detection (HPAEC/PAD) using a DIONEX ICS3000 chromatograph and CarboPac PA-1 (2×250 mm). The carbohydrate separation was performed using a multi-step gradient obtained after mixing eluent A (water) and eluent B (250 mM sodium hydroxide): 0 to 15 min, 100 mM; 15.1 to 20 min, 200 mM; and 20.1 to 25.5 min, 100 mM. The applied PAD potentials for E1 (0 to 0.4 s), E2 (0.41 to 0.42 s), E3 (0.43 s) and E4 (0.44 to 1.00 s) were 0.1, 2.0, 0.6 and 0.1, respectively, and the flow rate through the column was 0.25 ml^{-1} min. The peaks were identified through comparison with authentic standards of Mio-inositol, Glucose, Fructose, Sacarose, Raffinose and Estaquiose, with 99% purity (Sigma) (Figure 1). The protein concentration was determined using the Bradford test (Bradford, 1976).

Animals

Balb/c male mice (25 to 30 g, 30 days old) were obtained from the Biotery at the University of São Paulo. The study was approved by the Ethics Committee on the Use of Animals of UFMA (Application number: 8608/2011-00).

MCF-7 cancer cells

The MCF-7 breast cancer cell line was obtained from the Cell Bank of Rio de Janeiro. The cells were cultured in flasks containing Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum (FBS) and maintained in a humidified incubator at 37°C under 5% CO_2. When confluent, the cells were detached by addition of 3 ml of 0.025% trypsin-ethylene diamine tetraacetic acid and incubated at 37°C in a humidified incubator under 5% CO_2 for 5 min. Subsequently, the cell suspension was transferred into a 15 ml polyethylene tube containing 12 ml of RPMI medium supplemented with 10% FBS, centrifuged at 800 *g* for 10 min at 18°C, and washed again. The cells were adjusted to a concentration of $1×10^5$ cells and resuspended in 100 µl of enzyme assay buffer (EAB; at a concentration of 10 mg/kg) or saline solution (0.9%) 2 h prior to the sensitization assay.

Sensitization protocol

The animals were randomly divided into 4 groups (n=6/group): the

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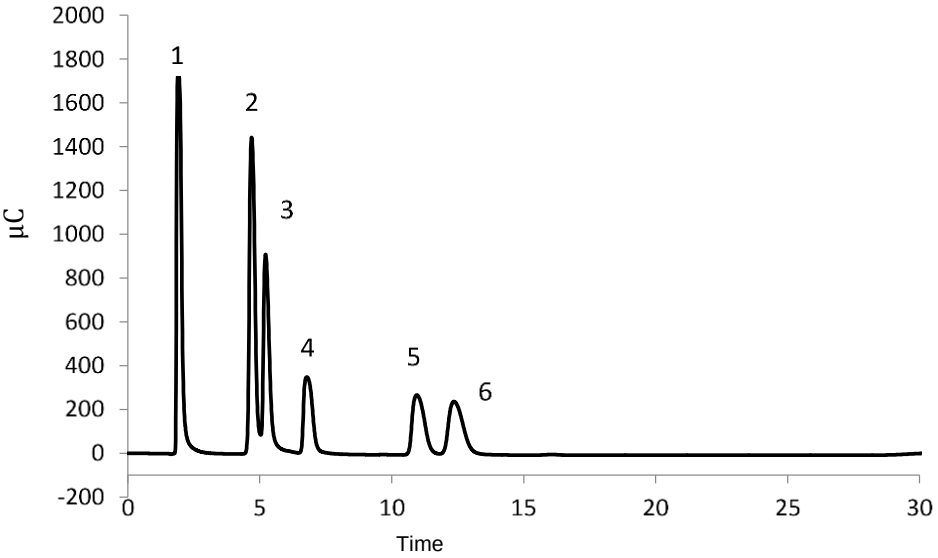

Figure 1. Separation of neutral and amino monosaccharides derived from glycoproteins. The peaks were identified by comparison with authentic standards of (1) Mio-inositol (2) Glucose (3) Fructose (4) Sacarose (5) Rafinose and (6) Estaquiose

control group in which the animals were sensitized with 100 µl sterile saline solution; the BME group in which the animals were inoculated with 100 µl BME (66,6 mg/kg); the tumor group in which the animals were inoculated with 100 µl MCF-7 cancer cell suspension at a concentration of 1×10^6 ml^{-1} in saline solution; and the tumor+BME group in which the animals were sensitized with a 100 µl MCF-7 cancer cell suspension at a concentration of 1×10^6 ml^{-1} in BME (66.6 mg/kg). Sensitization was subcutaneously performed 3 times on the dorsum, at an interval of 5 days. On the 15th day, the animals were euthanized through intraperitoneal access with an excess of anesthetics, including 2% xylazine hydrochloride (20 mg/kg), and 5% ketamine hydrochloride (25 mg/kg). Blood, bone marrow cells, and splenocytes were collected.

Blood collection and leukocyte count

The blood was collected in a micro hematocrit capillary tube by puncturing the orbital plexus. The blood was collected after adding HEMSTAB EDTA anticoagulant and leukocyte (white blood cells, WBC) count was determined using the hematology automatic analyzer.

Collection and counting of bone marrow cells

To collect bone marrow cells, the femur was removed and perfused with 1 ml phosphate buffered saline (PBS) solution. To count the cells, 90 µl of the suspension was mixed with 10 µl of 0.1% crystal violet solution. Subsequently, the cells were counted under an optical (light) microscope in a Neubauer chamber.

Cell collection and splenocyte count

The spleen was surgically removed, weighed, and homogenized in 3 ml PBS. The suspension was stored on ice until counting. For quantification, 90 µl of cell suspension was mixed with 10 µl of crystal violet, and the cells were counted under an optical (light) microscope in a Neubauer chamber. The splenocytes were resuspended in RPMI medium supplemented with 10% FBS at a concentration of 1×10^6 ml^{-1} and stored in a Petri dish for 2 h in a CO_2 incubator. After incubation, non-adherent cells were removed for phenotype characterization and co-culture experiments.

Phenotype characterization of splenocytes

The phenotypes of adherent and non-adherent cells, isolated from the spleen, were characterized with commercial monoclonal antibodies (BD Biosciences, San Jose, CA), according to the manufacturer's instructions. Two panels of antibodies were used, one for adherent cells, including anti-CD14 (FITC), anti-IA/IE (PE), and anti-Ly6G (PE-Cy5), and the other for non-adherent cells, anti-CD3 (FITC), anti-CD19 (PE-Cy5), anti-CD4 (PE), and anti-CD8 (PE-Cy5). After the acquisition of 10,000 events in a FACSCalibur flow cytometer, the obtained data were analyzed using FlowJo software.

Establishment of a co-culture with MCF-7 cancer cells

For co-culture experiments, MCF-7 tumor cells (1×10^4/well) were seeded in 96-well microtiter plates (Corning Costar) in RPMI medium supplemented with 10% FBS. After 24 h, non-adherent splenocytes were seeded at a concentration of 3×10^5 cells/well in RPMI medium supplemented with 10% FBS. After three days, the splenocytes were used for proliferation assays and phenotype characterization, and the supernatant was used to determine cytokine production.

Lymphoproliferation assay

To evaluate cell proliferation, the splenocytes were labeled with carboxyfluorescein succinimidyl ester (CFSE) using the CellTrace

CFSE cell proliferation kit according to the manufacturer's instructions and subsequently added to co-culture plates containing MCF-7 cancer cells. After three days, 10,000 events were acquired using a FACSCalibur flow cytometer. The proliferation capacity was determined as the decay of the average fluorescence intensity of the population corresponding to splenocytes, according to the Side and Forward Scatter (SSC and FSC, respectively) obtained using FlowJo software. The proliferation index was calculated as the ratio between the mean fluorescence intensity (MFI) of CFSE obtained in control splenocytes and the MFI of CFSE obtained in the other splenocytes.

Phenotype characterization of splenocytes after co-culture

The phenotype of the non-adherent splenocytes in co-culture was characterized using the commercial monoclonal antibodies (BD Biosciences, San Jose, CA) anti-CD3 (FITC), anti-CD4 (PE), and anti-CD8 (PE-Cy5) according to the manufacturer's instructions. After the acquisition of 10,000 events using a FACSCalibur flow cytometer, the obtained data were analyzed using FlowJo software.

Cytokine quantification by flow cytometry

The quantification of IL-2, IL-6, and IL-10 cytokines in the supernatant of the co-culture was performed using the Cytometric Beads Array (CBA) commercial kit according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using Graph Pad Prism version 5.0 software with Student's *t*-test and Newman-Keuls post hoc ANOVA test. Statistical significance was determined at a value of $p<0.05$. The data are expressed as the average±standard deviation of 6 animals per group.

RESULTS

The babassu mesocarp aqueous extract was rich in carbohydrates

The babassu mesocarp aqueous extract showed a yield of 75.54%. The aqueous extract showed a total sugar concentration of 29.79 mg/ml, with positive results for the presence of monosaccharides, reducing sugars, aldoses and ketoses, confirming the presence of polysaccharides. HPAEC analysis identified glucose, sucrose and fructose (Figure 2). The protein concentration in the aqueous extract was 0.506 mg/ml.

Sensitization with tumor cells altered the balance between T and B lymphocytes of the spleen

After the sensitization of Balb/c mice, the numbers of cells in the blood, spleen, and bone marrow were determined. The data showed that sensitization did not alter the number of circulating leukocytes and bone marrow cells in the different groups. However, the animals in the Tumor group showed an increase in spleen weight compared with the control group (Table 1).

Phenotyping of the cellular population of the spleen revealed that the frequency of B cells was higher in the tumor group compared with the other groups (Figure 3A). In contrast, the frequency of T cells was lower in the tumor group compared with the other groups (Figure 3B). The decreased frequency of T cells in the tumor group was followed by a reduction in the frequency of CD4+ T cells (Figure 3C). However, the frequency of CD8+ T cells in the tumor group was similar to that in the other groups (Figure 3D). No changes were observed in the frequency of CD14+, IA/IE+, and Ly6G+ splenocytes in any of the groups analyzed (data not shown).

Lymphoproliferative activity of splenocytes co-cultured with MCF-7 cells

To evaluate whether sensitization interferes with the proliferative capacity of splenocytes, non-adherent cells, and primarily represented by lymphocytes, were co-cultured with MCF-7 cancer cells. The data showed that splenocytes obtained from the BME group presented 3 times higher proliferative activity compared with the control group. No significant difference was observed in the tumor+BME group compared with the control group; however, significantly higher cell proliferation was observed compared with the tumor group (Figures 4A and B).

Phenotype characterization of splenocytes after co-culture with MCF-7 cancer cells

Splenocytes and MCF-7 tumor cells were co-cultured and the phenotypic analysis of T cells was performed. The results showed that the percentage of CD4+ T lymphocytes was lower in the tumor group compared with the other groups (Figure 5A). The results obtained in the tumor+BME group were similar to those obtained in the control group. However, no changes in cytotoxic T cells were observed (Figure 5B).

Cytokine production and release in the co-culture supernatant of splenocytes and MCF-7 cancer cells

The supernatant of the co-culture was removed to determine cytokine production. The data revealed that the concentrations of IL-6 and IL-10 were lower in the BME group compared with the control group. However, the tumor group showed significantly higher levels of IL-2, IL-6 and IL-10 than the control and BME groups. The splenocytes obtained from animals in the tumor+BME

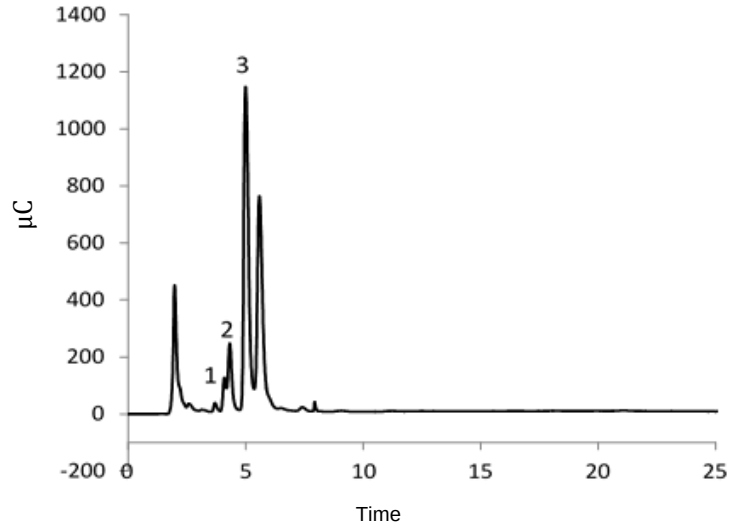

Figure 2. HPAEC chromatography identified of carbonhydrate the aqueous extract obtained from babassu mesocarp. HPAEC analysis identified type [(1) glucose, (2) fructose, and (3) sucrose in aqueous extract babassu]. The extract sample was deionizated in cationic-exchange column (Dowex 50W X 8-100)and anionic-exchange column (Dowex 1X 8-100). After, the sample was filtered through 0.45 µM membrane filtres. Samples with glucose equivalent (400 µg/ml) was analysed by anion exchange chromatography coupled with pulsed amperometric detection (HPAEC/PAD) using DIONEX ICS3000 chromatogram and carboPac PA-1 (2×250 mm). The carbonhydrate separation ws performed by using a multi-step gradient by mixture eluent A (water) and eluent B (250 mM sodium hydroxide): 0-15 min, 100mM; 15.1-20 min, 200 mM; and 20.1-25.5, 100 mM. the applied PAD potential for E1 (0-0.4 s) E2 (0.41-0.42 s), E3 (0.43 s) and E4 (0.44-1.00 s) were 0.1, 2.0. 0.6 and 0.1, respectively, and the flow rate through the column was 0.25 ml/min. The peak were identified by comparism with authentic standards of Mio-inositol, Glucose, Fructose, Sacrose, Rafnose, and Estaquiose with 99% purity Sigma.

Table 1. Immunological parameters.

Parameter	Groups (n=6/group)			
	CTL	BME	Tumor	Tumor+BME
WBC/µL (10^3)	7.8±1.7	6.2±1.4	6±1.7	5.8±1.1
Bone marrow cells (10^5)	58.1±7	52.6±5	61.4±8	65.3±7
Spleen weight (mg)	90±13	90±16	140±19*	110±13
Splenocytes (10^5)	1.4 ±304	1.4±216	1.7±436	1.2 ±208

The results are expressed as the average ± standard deviation of the total leukocyte count in the blood (WBC), number of bone marrow cells and weight and number of splenocytes obtained from groups sensitized with saline solution (Control group), babassu mesocarp extract (BME) alone (BME group), MCF-7 cancer cells resuspended in saline solution (Tumor group), and MCF-7 cancer cells resuspended in BME (Tumor+BME group). The results were analyzed using the Newman-Keuls post hoc ANOVA test, and * indicates $p < 0.05$ compared with the Control group (n=6/group).

group did not show a significant difference in cytokine production compared with the other groups, but there was a decrease in the IL-6 and IL-10 concentrations compared with the tumor group (Table 2).

DISCUSSION

In the present study, Balb/c mice were subcutaneously sensitized with MCF-7 cancer cells, which are ideal for

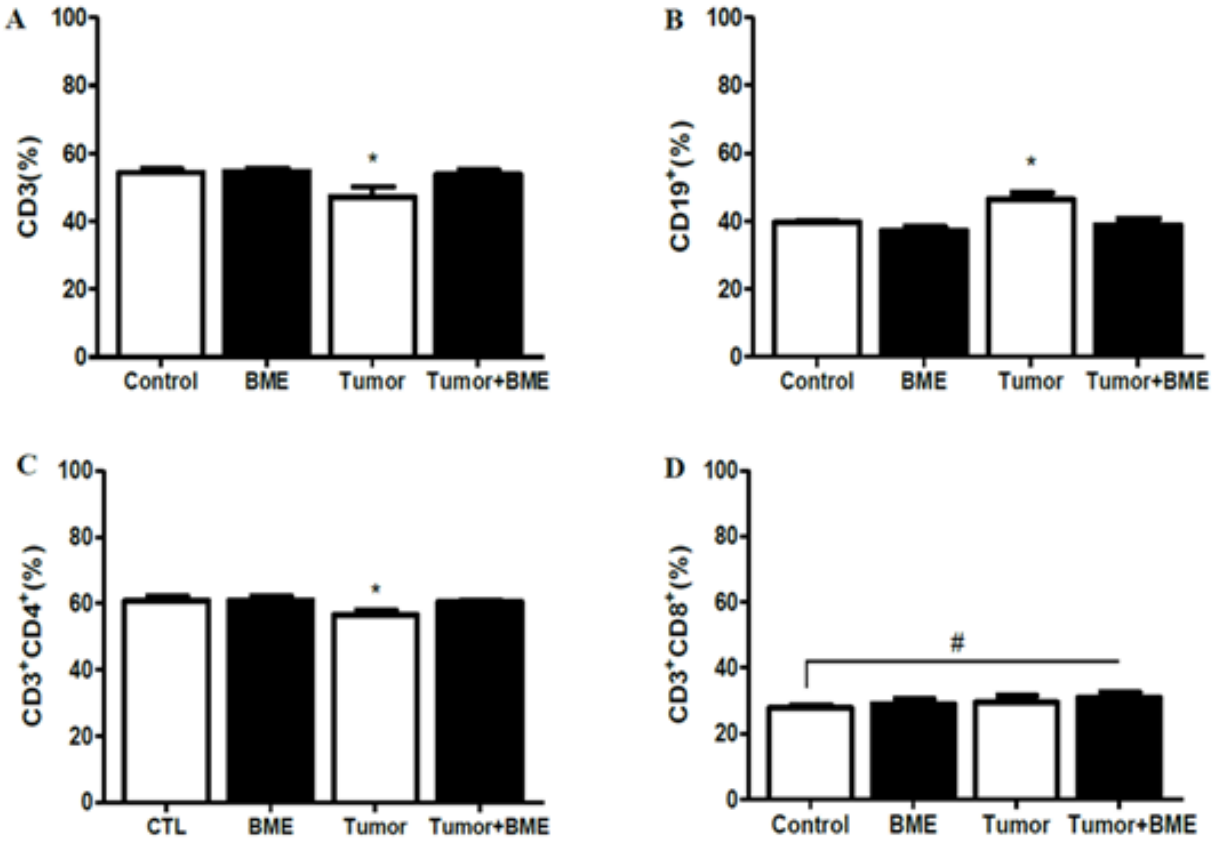

Figure 3. Phenotyping of splenocyte cultures obtained after sensitization. Frequency of T cells (A), B cells (B), and subpopulations of T helper (C) and cytotoxic T cells (D), respectively, were analyzed in the spleen of animals after subcutaneous sensitization with saline solution (Control group), extract rich in carbohydrates obtained from Babassu mesocarp extract (BME) alone (BME group), suspension of MCF7 cancer cells in saline solution (Tumor group), or suspension of MCF7 cancer cells in BME (Tumor+BME group). Values are expressed as average ± standard deviation and analyzed by the Newman-Keuls post-hoc ANOVA test with *$p<0.05$ compared with all groups and $^{\#}p<0.05$ compared with a single group.

antigen recognition by resident immune cells and subsequent migration and presentation to secondary lymphoid organs. In the context of immunization with tumor cells, the immune system might experience two types of naive responses: the low capacity of the host to trigger local inflammation that might compromise tumor antigen presentation, and poor immunogenicity in response to the tumor (Edelman and Jefford, 1968). Balb/c mice induced an adequate response after immunization (Crowther and Wasgstaff, 1983; Ochsenbein et al., 2001; Ochesenbein, 2002). Although these cells do not proliferate in mice, upon sensitization, human MCF7 cancer breast cancer cells induce the proliferation of splenocytes and after fusion, form hybridomas that produce specific monoclonal antibodies (Ochsenbein et al., 2001; Ochsenbein, 2002; Schunck and Macallum, 2005).

In the present study, the results showed no significant differences in the leukocyte number and cellularity of the lymphoid organs. However, increased spleen weight and a lower frequency of T helper cells were observed in animals sensitized with MCF-7 cells. These data contradict the expectation, as the inoculation of human cells in mice should lead to a xenograft reaction involving cell rejection and T lymphocyte stimulation (Mandal-Ghosh et al., 2007; Menard et al., 1983). However, studies with athymic mice showed that the overexpression of transforming growth factor beta (TGF-β) induces immunosuppression in the MCF-7 cancer cell line (Sun et al., 1992; Arteaga et al., 1993; Sachs, 1995; Schunk and Macallum, 2005; Koch et al., 2013). Although, the thymus of the animals was maintained in the present study, we suggest the existence of potential mechanisms that might be involved in the steps occurring between xenograft rejection and tumor cell escape, presumably affecting the T helper cell response.

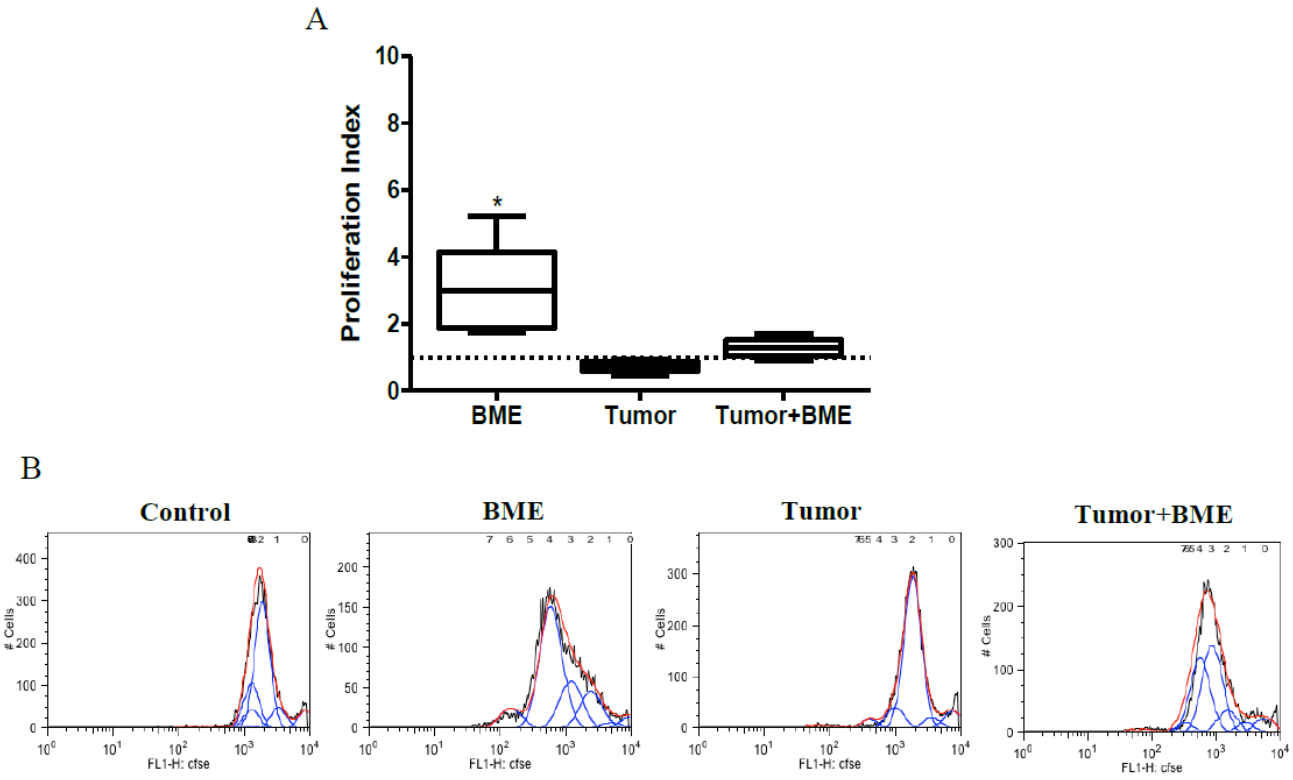

Figure 4. Proliferation of splenocytes after sensitization and maintainance in culture with MCF7 cancer cells. The proliferation rate was calculated from the ratio between the mean fluorescence intensity (MFI) of carboxyfluorescein succinimidyl ester (CFSE) in the control group sensitized with saline solution and the other groups by determining the number of times that the group sensitized with Babassu mesocarp extract (BME) alone (BME group), MCF7 cancer cells (Tumor group), or MCF7 cancer cells and BME (Tumor+BME group) promotes cell division compared with the control (dashed line) (A). Histograms for the different groups illustrate CFSE fluorescence intensity; lines inside the peak indicate the shift of CFSE MFI to the left, which is proportional to increased cell proliferation (B). Values are expressed as average ± standard deviation and analyzed by the Newman-Keuls post-hoc ANOVA test with * indicating $p<0.05$ compared with all groups.

However, the frequency of CD3+CD4+ cells in the group sensitized with MCF-7 cancer cells and BME was similar to that in the control group. Although the difference was not significant, sensitization with babassu mesocarp, with or without MCF-7 cancer cells, increased the frequency of IA/IE$^+$ cells, demonstrating the occurrence of improved antigen presentation, the modulation of the resident cells' pro-inflammation response (Azevedo et al., 2003), or the generation of complexes with tumor proteins that increase immunogenicity (Richmond and Su, 2008; Ju et al., 2001). Nascimento et al. (2006) showed that in animals intraperitoneally treated with babassu mesocarp, the migration of cells into the peritoneum increased, primarily through the activation of macrophages that release hydrogen peroxide (H_2O_2) and nitric oxide (NO). This finding indicates that babassu mesocarp promotes an M1 type response. M1 macrophages are activated through classical pathways and play an important role in eliminating tumor cells via the production of high levels of pro-inflammatory cytokines that lead to a Th1 response (Koch et al., 2013; Schunk and Macallun, 2005; Richmond and Su, 2008; Sachs, 1995; Arteaga et al., 1993; Sun et al., 1992). Using isogenic C57Bl/6 and Balb/c mice, Fortes et al. (2009) showed antitumor activity and improved survival of animals sensitized with solid and ascitic Ehrlich tumors. In the present study, in addition to the recovery of T helper cells, sensitization with BME and tumor cells increased the frequency of cytotoxic T cells and reduced the frequency of B cells compared with animals sensitized with the tumor alone. These data reinforce the idea that the carbohydrates of babassu mesocarp might exhibit immunomodulatory activity through the induction of a cytotoxic response.

In the proliferation assay, the group sensitized with MCF-7 cancer cells did not present any differences compared with the control group, and the population of T helper cells remained lower after co-culture. These data reinforce the hypothesis that the presence of a tumor

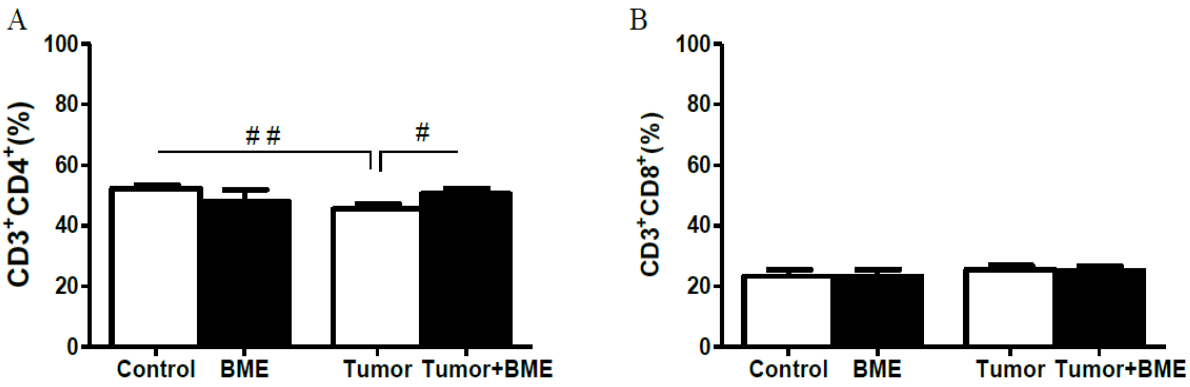

Figure 5. Phenotyping of splenocytes obtained after sensitization and co-cultured with MCF-7 cells. Frequency of T helper cells (A) and cytotoxic T cells (B) were analyzed 3 d after co-culturing MCF-7 tumor cells with non-adherent splenocytes obtained from animals sensitized subcutaneously with saline solution (Control group), Babassu mesocarp extract (BME) rich in carbohydrates (BME group), suspension of MCF-7 tumor cells in saline solution (Tumor group), or with the suspension of MCF-7 tumor cells in BME (Tumor+BME group). Values are expressed as average ± standard deviation and analyzed by Newman- Keuls post-hoc ANOVA test with $^{\#}p<0.05$ and $^{\#\#}p<0.01$ compared with a single group.

Table 2. Cytokine concentrations in the supernatant of splenocyte and MCF-7 tumor cell co-cultures.

Parameter	Groups			
	Control	BME	Tumor	Tumor+BME
IL-2 (pg/ml)	0.5±0.2	0.05±0.1	14.5±5.7*	3.5±3.7
IL-6 (pg/ml)	122.9±3.0	32.0±14.3*	363.1±172.8*	194.4±128.9
IL-10 (pg/ml)	89.1±7.6	14.8±12.2*	115±54.8*	78.2±38.6

The results are expressed as the average of the duplicates ± standard deviation of interleukins (IL) 2, 6, and 10 concentrations in the supernatant of splenocyte and MCF-7 cancer cell co-cultures obtained from groups sensitized with saline solution (Control group), BME alone (BME group), MCF-7 cancer cells resuspended in saline solution (Tumor group), and MCF-7 cancer cells resuspended in BME (Tumor+BME group). The results were analyzed using the Newman-Keuls post hoc ANOVA test, and *p < 0.05 compared with the Control group (n=6/group).

might activate immunosuppressive mechanisms that could affect effector lymphocyte ability. However, the proliferation rates of the splenocytes obtained from the BME and tumor+BME groups were higher, suggesting that BME treatment induced the immune response against tumor-associated immunosuppression.

The data show that increased levels of IL-2, IL-6, and IL-10 were observed in the supernatant of the splenocyte co-culture obtained from animals of the tumor group, thus indicating a regulatory response. The splenocytes obtained from the animals of the tumor+BME group showed a decrease in all cytokines, similar to the control group. The data reinforce a Th1 adjuvant role for the carbohydrates extracted from babassu mesocarp, as the entire response induced in Balb/c mice stimulated with MCF-7 cancer cells was reversed when the tumor stimulus was associated with BME.

The mesocarp of the babassu fruit is rich in carbohydrates and in particular, a mucopolysaccharide, MP1, which was shown to induce phagocytic activity (Silva and Parente, 2001). Other studies demonstrated that treatment with mesocarp exerts immunoregulatory effects. Nascimento et al. (2006) showed that intraperitoneal injections of BME increased cellular migration to the peritoneal cavity, promoted the activation of peritoneal macrophages both *in vitro* and *in vivo*, and increased the expression of major histocompatibility complex (MHC) class II and the recruitment of immune cells (Nascimento et al., 2006). Fortes et al. (2009) showed that BME exerted an immunomodulatory effect, resulting in increased survival of the animals and a decreased size of the solid Ehrlich tumors when this tumor cell line was pretreated with BME. Guerra et al. (2011) demonstrated that immunization with promastigote forms of *Leishmania amazonensis* and the addition of BME induced a Th1-dependent immune response in

Balb/c mice, thus showing promising effects compared with other adjuvants. Despite their poor immunogenicity, the ability of carbohydrates to exert an immunomodulatory effect through the formation of complexes with tumor proteins potentiates immunospecific responses. This is the case for the oligosaccharides in Lewis Y-containing glycoproteins, which are used in mice to form complexes that induce the production of antibodies that exert cytotoxic effects against MCF-7 cells through the activation of macrophages (Kudryashov et al., 1998; Silva and Parente, 2001; Mantovani et al., 2004) by the binding of these molecules to membrane receptors (Um et al., 2002; Moretão et al., 2003; Ma et al., 2010) or the increase in the host immune response against tumors, which enhances leukocyte action and cytokine production (Mosser, 2003). The results showed that BME induces cell proliferation, increases T helper cells and reduces IL-6 and IL-10 levels, thereby reversing tumor-associated immunosuppression.

Therefore, this study showed that BME modulates the frequency of T helper cells and B-lymphocytes in the spleen of Balb/c mice after sensitization with human MCF-7 cancer cells. Moreover, *in vitro* experiments demonstrated that BME improved the lymphoproliferative activity and modulation of cytokines in the presence of MCF-7 cancer cells. These data reinforce the immunomodulatory effect of the carbohydrates extracted from babassu mesocarp, thus demonstrating an adjuvant role in the development of antitumor vaccines.

Conflict of Interests

The authors have not declared any conflicts of interests.

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