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Mechanism Anti-Tumor of IgA-based Delivery System on the Human Colostral Mononuclear Cells via Fcα Receptor

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Abstract: This study investigated the effect of anti-tumor of the IgA-based delivery system on the human colostral mononuclear cells via the Fcα receptor co-cultured with breast cancer cell lines (MCF-7-ATCC) and the colostrum mononuclear (MN) cells were collected from healthy mothers'.. MN cells and co-culture (MN plus MCF-7 cells) were pre-incubated for 24 h with or without 100 ng. mL^{-1} secretory immunoglobulin A (SIgA), PEG microspheres (PEG), or 100 ng. mL^{-1} SIgA adsorbed to PEG microspheres (PEG-SIgA). Cells subsets of MN cells, chemokines (colostrum or culture supernatant), and apoptosis in the MN cells and co-culture were determined by flow cytometry. The highest percentage of mononuclear cells present in the colostrum was macrophages; approximately 80% of the cells expressed CD14+. There was an increase in the release of MIG in the co-culture of MN cells and MCF-7 cells and reduced IL-8 and MCP-1 in the presence of PEG-SIgA. The MN cells in co-culture with the MCF-7 cells, independent of treatment, showed the highest apoptosis index. The addition of a MoAb anti-human Fcα receptor resulted in a significant increase of IL-8 and MCP-1 release in co-culture and higher apoptosis rates, suggesting an interaction between sIgA and FcαR mechanism anti-tumor of IgA-based delivery system. These data suggest that the control of chemokines release and apoptosis in co-culture incubated of PEG-SIgA may be one alternative mechanism involved in protection against the breast tumor.

Keywords: SIgA; PEG microsphere; MN cells; Breast Cancer; FcαRI.

1. Introduction

Antibodies (Abs) can exhibit direct (Fab-mediated) and indirect (Fc-mediated) anti-tumor effects. Human breast milk presents large amounts of secretory IgA (SIgA) antibodies [1-4], which play a protective role against several diseases. SIgA from the human colostrum has anti-tumor effects in human MCF-7 breast cancer cells [5], and this activity is important because this protein is part of the primary antibody class in human breast milk [4, 6]. This protein secreted by breast milk may protect against breast tumors in women who breastfed [5].

In addition to SIgA effectors, immune cells may contribute to the antibody's efficacy as protection against tumor growth. During the past decade, insights have been gained regarding the mechanisms underlying the dynamic interplay between immune cells and tumor progression [7, 8]. Human colostrum contains large amounts of viable leukocytes. Colostrum cells are constituted by polymorphonuclear (PMN) cells, among them neutrophils and

mononuclear (MN) cells that are lymphocytes and macrophages [6, 9]. The major mononuclear cells present in the colostrum are macrophages [10].

Colostral macrophages may be activated by stimulatory signals generated by milk antibodies, especially during SIgA interaction with its Fc receptor (FcαR or CD89) [9-13]. Some studies have reported that interactions between IgA and FCαR have been identified as a candidate target for tumor therapy, leading to tumor cell killing [14]. Still, the effects of anti-tumor of Secretory Immunoglobulin A (SIgA) interaction with its specific Fcα receptors on colostral phagocytes need further investigation. Considering a large amount of SIgA and mononuclear cells in the colostrum, especially macrophages with receptors for SIgA [9], it is possible that interactions between cells and antibodies may activate tumoricidal mechanisms that contribute to the effects protective against breast cancer that are conferred for the mother during breastfeeding.

However, protein-based drugs, including antibodies, are being developed and may represent an innovative therapeutic. Polyethylene glycol (PEG) microspheres are polymeric particles that can absorb organic compounds and are considered a major drug carrier [15]. PEG microspheres are promising because they prevent the degradation of the substances adsorbed and increase their bioavailability in the organism, thereby modulating the immune system [16, 17]. PEG combined with colostral SIgA has been used to reduce cell viability and induce apoptosis in breast cancer cell lines (MCF-7 -5).

On the other hand, several chemokines have been reported in human milk. Because of the various signaling pathways, chemokines have multifaceted functions and play an important role in regulating immune surveillance, wound healing, tissue repair and inflammation [18], and tumor metastasis and tumor cell spread [19]. So the release of modified IgA may act on the signaling of colostrum cells modulating these proteins and key functions of these cells, potentiating the immune responses and anti-tumor activity. Nevertheless, no studies have linked interactions between SIgA adsorbed to PEG microspheres and colostral mononuclear cells by Fcα receptor. This study aimed to investigate the effect anti-tumor of the IgA-based delivery system on the human colostral mononuclear cells via the Fcα receptor co-cultured with breast cancer cell lines (MCF-7).

2. Materials and Methods

2.1. Subjects.

This cross-sectional study evaluated 30 mothers clinically healthy at the Health System Program of Barra of Garças, Mato Grosso, Brazil. Before entering the study, the volunteers signed an informed consent form approved by the local ethics committee Araguaia of the Federal University of Mato Grosso (Protocol Number CAAE: 45102815.3.0000.5587).

2.2. Obtaining supernatants from colostrum.

About 8 mL of colostrum from each woman were collected in sterile plastic tubes between 48 and 72 hours postpartum. The colostrum samples were centrifuged (160 x g, 4°C) for 10 min. The aqueous supernatant was stored at -80ºC for obtaining purified SIgA and chemokines determinations and the reserved cells for later analyses.

2.3. Colostral purified SIgA.

Human colostrum SIgA was purified from colostrum pool by affinity chromatography on Cyanogen Bromide-Activated Sepharose-4B (CNBr-Sepharose-4B - Sigma, ST Loius, USA) bound with sheep anti-human α chain as proposed by March et al. [20]. After the concentration of SIgA was determined by simple radial immunodiffusion. The preparation was also tested by immunoelectrophoresis with goat anti-human γ and μ chain antisera [8]. IgG and IgM antibodies were undetectable in the preparation. SIgA purified was 3.4 g/L adjusted to a concentration of 100 ng/mL. The aliquots were stocked at -80oC and subsequently used for experiments.

2.4. Polyethylene glycol (PEG) microsphere preparation.

The microspheres were obtained from polyethylene glycol (PEG) 6000 using a modification [16] of a previously described protocol [21]. In brief, 20 g of PEG 6000 was diluted in 100 mL of a 2% sodium sulfate solution in phosphate-buffered saline (PBS) and incubated (37°C; 45 min). The PEG microspheres were diluted in 3: 1 proportion in PBS, washed twice in PBS (500 x g, 5 min), and resuspended in PBS. The microspheres were thermally induced, placing the solution at 95°C for 5 min. The adsorption was performed by incubating PEG microspheres' suspensions in PBS with SIgA (Sigma, St. Louis, USA; concentration 100 ng mL-1) at 37°C during30 min. The PEG microspheres with adsorbed SIgA were washed twice in PBS (500 x g, 5 min). The loading efficiency of adsorption of the PEG microspheres with adsorbed secretory IgA (SIgA) was confirmed by fluorescently labeled overnight at room temperature with a solution of Dylight-488 (Pierce Biotechnology, Rockford, USA; 10 μg mL-1) in dimethylformamide (100:1 molar ratio of PEG: Dylight) and analyzed by fluorescence microscopy [5].

2.5. Separation of colostral cells.

Colostrum was collected in plastic tubes and centrifuged (160 x g, 4°C) for 10 min. Ficoll-Paque gradient (Pharmacia, Upsala, Sweden) used for cell separation. This procedure generated 98% pure mononuclear cell preparations as analyzed by light microscopy [22]. Purified macrophages were resuspended independently in serum-free 199 medium at a final concentration of $2x10^6$ cells/ml. After this period, the macrophages were washed twice and used by assay.

2.6. MCF-7 cell culture.

MCF-7 human breast cancer cells (American Type Culture Collection ATCC, USA). The cells were maintained as monolayer cultures (75 cm^2 plastic culture flasks) in RPMI 1640 medium with 10% fetal bovine serum (FBS-Sigma, St. Louis, MO, USA), penicillin (20 U/mL), and streptomycin (20 μg/mL-Sigma, St. Louis, MO, USA) at 37°C in an incubator with an atmosphere containing 5% CO_2. The cells were cultured every 5±2 days.

2.7. Co-culture of MN cells and MCF-7 cells treatment.

To explore the cell subset, chemokines levels, and apoptosis induction (annexin V staining), subconfluent (80%) monolayers of MCF-7 cells were trypsinized (Sigma, St. Louis, USA) adjusted for $5x10^6$ cells/mL. Then, the trypsinized cells were pre-incubated with MN

cells [23] for 24 h with or without 50 µL at 100 ng/mL final concentration of SIgA (100 ng/mL final concentration), polyethylene glycol (PEG) microspheres or SIgA adsorbed in PEG microspheres (PEG-IgA-100). After, the cells were diluted in RPMI 1640 medium with 10% fetal bovine serum (FBS -Sigma, St. Louis, MO, USA), penicillin (20 U/mL-Sigma, St. Louis, MO, US), and streptomycin (20 µg/mL-Sigma, St. Louis, MO, USA) at 37°C for 24 h in a humid atmosphere containing 5% CO_2. After this period, the cell co-cultures were washed twice and used for analyzes.

2.8. MN cells treatment.

Cell subset, chemokines levels in culture supernatant and apoptosis induction (annexin V staining) colostral MN cells were pre-incubated with or without 50 µL of SIgA (100 ng/mL final concentration), of polyethylene glycol (PEG) microspheres (50 µL) and SIgA adsorbed in PEG microspheres (PEG-IgA, 50 µL-100 ng/mL final concentration) during 24 hours. The cells were resuspended in medium (RPMI 1640) contained 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO, USA), penicillin (20 U/mL-(Sigma, St. Louis, MO, USA), and streptomycin (20 µg/mL- Sigma, St. Louis, MO, USA) at 37°C during 24 h in a humid atmosphere containing 5% CO_2.

2.9. Cell subsets.

After the culture with the different treatment, the colostrum cells in co-culture or not were stained with 5µL of anti-CD3; anti-CD4FITC, anti-CD8PE, anti-CD14FITC, and anti-CD14FITC at room temperature for 30 min. All cells were washed and resuspended in phosphate-buffered saline (PBS) with bovine serum albumin (BSA- Sigma, St Louis, USA; 5mg/mL) for flow cytometry analyses. Isotypic controls (IgG1-FITC, IgG1-PE both from BD Biosciences). A minimum of 10.000 cells was gated by size and granularity by flow cytometer (FACSCalibur, BD Biosciences, USA), and data analyzed by the Flowjo 7.2.5 software. 4.4.5.

2.10. Colostral mononuclear cells with MoAb anti-human Fcα receptor treatment.

To assess the effect of SIgA-FcαRI interaction on the anti-tumor activity of mononuclear phagocytes ($2x10^6$ cells/mL) were incubated with 50 µL of a MoAb reactive with human FcαRI (0.1mg/mL- Medarex, Inc., West Lebanon, NH), monoclonal Ab that binds in the EC1 domain of FcαRI can block IgA binding [13] for 2 h at 37ºC. They were then washed once with medium 199 at 4ºC and immediately used in the chemokines levels and apoptosis induction.

2.11. Quantification of chemokines in colostrum or supernatant of the culture of MN.

Chemokines Interleukin-8 (CXCL8/IL-8), chemokine monokine induced by γ-interferon (CXCL0/MIG), monocyte chemoattractant protein-1 (CCL2/MCP-1), and protein 10 (CXCL10/IP-10) concentrations in colostrum supernatant or the supernatant of cultures of MN cells treated or not with a MoAb reactive with human FcαRI, MCF-7 cells and co-culture (MN and MCF-7 cells) were measured with cytometric bead array (CBA, BD Biosciences, USA) according to the manufacturer procedures (CBA, BD Biosciences, USA). A flow cytometer (FACSCalibur, BD Biosciences, USA) was used for analysis; cytometric graphs

were generated using CellQuest (BD Biosciences, USA) software, and the data were obtained using 1.0 FCAP Array (BD) software.

2.12. Apoptosis assay.

The apoptosis rates were verified by the Annexin V staining. As negative controls, the untreated cells were used, and cells treated with staurosporine (Sigma, St. Louis, USA), which was used to induce apoptosis, were used as positive controls. The cells (MN cells treated or not with a MoAb reactive with human FcαRI or co-culture with MCF-7) were diluted in 500 µL of the binding buffer with 5 µL of annexin V-FITC (Alexis, San Diego, USA) and 5 µL of PI and then incubated for 10 min at room temperature. The cells' fluorescence was analyzed by flow cytometry (FACSCalibur system; BD, San Jose, USA). The data were analyzed using CellQuest software, and the cells were classified in viable cells (annexin$^-$/PI$^-$), early apoptotic cells (annexin+/PI$^-$), late apoptotic cells (annexin+/PI+), and necrotic cells (annexin$^-$/PI+).

2.13. Statistical analysis.

We utilized analysis of variance (ANOVA) was used to evaluate subsets cells, chemokines, and apoptosis index. Were considered statistical significant Statistical when $p<0.05$.

3. Results and Discussion

The combination of antibodies and polymeric matrices has been developed as an alternative therapy for various diseases, between then cancer. In the present study, SIgA antibody adsorbed to PEG microspheres increased colostral MN cells' anti-tumor activity against MCF-7 human breast cancer cells via the Fcα receptor.

The modified delivery system can maintain an adequate period until the protein arrives at the site of action and is released in a controlled manner [24]. PEG microspheres act by extending the drug's relative bioavailability compared with that of the free drug and potentiating the pharmacological action [25-27].

For sought to investigate whether changes in cellular subsets could be associated with the anti-tumor activity. The cells treated with SIgA and PEG-SIgA in co-culture with the MCF-7 cells increased CD3$^+$ T cells' percentage. An increase in CD8$^+$ T cells when these cells were co-cultured with MCF-7 cells and treated with PEG-SIgA (Table 1). Regardless of treatment or the presence of tumor cells, the highest percentage of mononuclear cells present in the colostrum was macrophages, which represented approximately 80% of cells expressing CD14$^+$ (Table 1).

Mucosal immunity is of great importance as a natural barrier, and SIgA acts as the first line of defense against microorganisms. Human breast milk is particularly rich in SIgA, and interactions of this antibody with cells of the secretion itself are extremely important to protect various diseases [4, 9, 12]. Several factors influence the distribution of cell types in milk. The study of milk cells provides a good cancer research model [28], especially breast cancer [7]. Although macrophages represent a major component of immune infiltrate in breast cancer and can contribute to tumor progression and dissemination, studies suggest that reprogrammation of these cells can act as a potent anti-tumor immune response [29]. Also, it should be considered that colostrum macrophages are cells that present morphofunctional differences with other

types of monocytes and macrophages present in other tissues, which can, independently of reprogramming, exert anti-tumor effects on breast cancer.

Table 1. Surface phenotypes of colostral mononuclear (MN) cells. Percentage of T cells ($CD3^+$), T helper cells ($CD4^+$), T cytotoxic cells ($CD8^+$), and macrophages ($CD14^+$) in the human colostrum after 24 hours in culture.

Culture	Subsets cells (%)			
	$CD3^+$	$CD4^+$	$CD8^+$	$CD14^+$
MN cell	15.7±5.6	8.2±1.6	4.4±2.9	80.3±7.8
MN cell+ PEG	14.6±2.5	8.1±2.4	6.2±3.4	81.5±9.7
MN cell+MCF-7	14.5±2.	9.0±2.6	7.2±3.8	79.9±5.9
MN cell+MCF-7+SIgA	17.5±1.8*	7.1±3.9	6.5±2.6	82.5±8.7
MN cell+MCF-7+PEG-SIgA	18.8±1.6*	8.3±2.7	9.3±1.2*	80.5±7.2

Notes: Polyethylene glycol (PEG) microspheres; secretory immunoglobulin A (SIgA); secretory immunoglobulin A adsorbed to PEG microspheres (PEG-SIgA). $P<0.05$ between treatments (PEG, SIgA, and PEG-SIgA) considers the same kind of cells' subsets.

Interestingly, the PEG-SIgA increased T cells expressing $CD8^+$ when in co-culture with MCF-7 cells. $CD8^+$ T lymphocytes are an important cell of the tissue microenvironment and can recognize and eliminate breast tumor cells [30[. Here, the increase of $CD8^+$ T lymphocytes by PEG-SIgA probably should be due to the activation and proliferation of these cells. Other studies should be performed to investigate the effects of PEG-SIgA on other isolated myeloid cells to clarify the anti-tumor mechanisms that involve the cells of the colostrum.

The diversity of the immunostimulatory molecules has been associated with anti-tumor effectors [31]. The chemoattractive properties of chemokines on different leukocyte subpopulations and their effects on the development of anti-tumor immune responses have been shown [32].

Chemokines (IL8, MIG, MCP-1, and IP-10) were assessed in the colostrum supernatant (Figure 1) and the supernatant of the culture of MN cells treated or not with a MoAb reactive with human FcαRI co-cultured or not with the MCF-7 cells and treated with PEG-SIgA (Table 2).

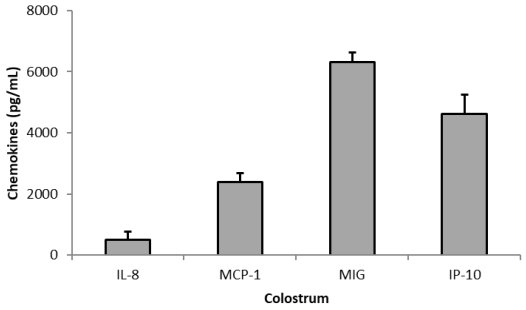

Figure 1. Chemokine concentrations in the colostrum supernatant of healthy mothers. Data presented as mean ± standard deviation (SD) of 15 colostrum samples of different mothers.

The colostrum supernatant showed a higher MIG and IP-10 and lower concentrations of IL-8 and MCP-1 (Figure 1). The level of IL-8, independently of treated of cells with a MoAb reactive with human FcαRI, was lower in the supernatant of the MN cells than in the supernatant of the MN cells co-cultured with MCF-7 cells. In culture or not with MCF-7, the MN cells incubated with SIgA or PEG-SIgA reduced the IL-8 level. The treatment of MN cells with MoAb reactive with human FcαRI increased the IL-8 level supernatant of culture when the cells were co-cultured with MCF-7 e incubated with PEG-SIgA. MN cells and MCF-7 cells' co-culture increased the MCP-1 levels, whereas the treatment with PEG-SIgA decreased this chemokine. MIG concentration was higher in the supernatant of the MN cells' culture treated

with SIgA or PEG-SIgA and co-cultured with the MCF-7 cells. Compared to untreated cells, MN cells pretreated with AbMo anti-FcαRI had lower MIG concentration in the supernatant of the culture incubated with PEG-SIgA. The co-culture of MN cells and MCF-7 cells, when incubated with PEG-SIgA, increased the IP-10 concentration, and the lower was observed when the cells were treated with monoclonal anti-FcαRI (Table 2).

Table 2. Chemokine concentrations in the supernatant of cultures of colostral MN cells treated with SIgA and PEG-SIgA co-cultured but not with MCF-7 cells after 24 hours in culture.

		Chemokines (pg/mL)			
MN Cells incubated	**Moab anti-FcαRI**	**IL-8**	**MCP-1**	**MIG**	**IP-10**
Medium	No	16.7±3.2	41.8±25.4	79.8±21.3	72.8±17.8
	Yes	16.2± 3.0	42.9± 21.0	70.9±14.1	75.3±22.0
PEG	No	16.2±2.9	34.8±5.8	71.7±9.4	69.4±18.1
	Yes	14.0±6.1	35.4±2.1	70.0±8.1	67.3±12.2
Siga	No	11.0±3.4*	46.2±25.0	66.2±9.6	101.6±25.9*
	Yes	26.6±3.2$^{\#}$	44.9±3.8	75.4±9.9	75.2±5.1$^{\#}$
PEG-SIgA	No	9.2±2.8*	55.2±5.8	83.2±10.3	59.6±4.5
	Yes	19.7±3.9$^{\#}$	30.4±12.2	35.8±9.9*$^{\#}$	27.8±4.4*$^{\#}$
MCF-7	No	26.4±7.4	102.2±5.7	46.6±3.9	46.6±18.6
	Yes	28.1±4.8	106.8±4.3	49.1±5.2	34.8±11.5
MCF-7+PEG	No	29.5±8.9	94.9±3.3*	53.1±3.4	44.3±4.9
	Yes	21.8±6.8	93.2±6.5*	50.7±6.9	46.0±2.2
MCF-7+SIgA	No	10.5±1.8*	36.9±10.4	95.4±6.9*	48.7±18.0
	Yes	18.9±3.4$^{\#}$	27.1±9.1	46.9±3.6*$^{\#}$	33.6±4.3*$^{\#}$
MCF-7+PEG-SIgA	No	4.9±1.3*	18.4±8.9*	101.9±14.4*	62.8±8.3*
	Yes	26.0±1.1$^{\#}$	24.2±6.2	38.8±1.1*$^{\#}$	38.7±2.7*$^{\#}$

Notes: The results represent the mean ± SD of six experiments. Polyethylene glycol (PEG) microspheres; secretory immunoglobulin A (SIgA); secretory immunoglobulin A adsorbed to PEG microspheres (PEG-SIgA). *p<0.05 untreated cells compared with treated cells (PBS). $^{\#}$p<0.05 indicates differences between Moab anti-FcαR use within each treatment.

Chemokines are proteins with low molecular weight chemotactic cytokines that present effects on cells [33]. Anti-tumor activity of MIG and IP-10 has been reported in various tumor models [34, 35]. Evidence suggests that these chemokines are also key to tumor inhibition mediated by other cytokines and chemokines [36-38]. However, MCP-1 and IL-8 have been associated with tumor progression [39, 40]. In this study, we showed the control of chemokine release in the presence of PEG-SIgA probably could contribute to the anti-tumor activity. Here, the high levels of MIG and IP-10 in colostrum associated with the secretion in co-culture modulated by SIgA via FcαR suggests an important mechanism anti-tumor of colostrum.

However, the balance between a protective secretory immune response and the degree to which the cellular and soluble components are present in human milk should promote reducing breast cancer, which depends on factors that can be regulated systematically by an immune response [5]. Studies have suggested that the low IgA production in patients with cancer may be an additional risk factor for tumor development [41]. Due to cancer's complicated pathogenesis and the versatile refractory of tumor cells, combinatorial strategies favor cancer therapies. Exogenous monoclonal antibodies to neoantigens have reduced tumors' growth by various mechanisms, including apoptosis [42, 43].

The apoptosis index in MN cells treated or not with MoAb reactive with human FcαRI and co-culture (MN and MCF-7 cells), cells were stained with annexin V and analyzed by flow cytometry (Table 3).

Table 3. Apoptosis (%) and necrosis (%)of colostral Mononuclear (MN) Cells and co-culture of colostral MN Cells and MCF-7 Cells in the presence of SIgA adsorbed to PEG microspheres.

MN Cells Incubated	MoAb anti-FcαRI	Viables Q1	Apoptosis Q2+Q3	Necrosis Q4
Medium	No	91.8±0.9	6.7±1.7	1.5±0.3
	Yes	89.1±0.4	9.9±2.9	0.1±0.05$^{\#}$
PEG	No	90.0±2.5	5.9±2.1	4.1±1.0*
	Yes	86.5±2.3	6.0±0.8	7.4±1.5*
SIgA	No	89.7±0.9	7.4± 1.3	1.9±0.1
	Yes	86.1±0.8	9.7±0.3	3.9±0.6*$^{\#}$
PEG- SIgA	No	89.1±1.3	9.6 ±2.3	2.2±0.5
	Yes	87.4±1.4	9.3±1.6	1.4±0.3
MCF-7	No	79.8±9.8	14.4±5.2	5.8±1.2
	Yes	80.7±2.3	15.1±1.1	3.9±2.1
MCF-7+PEG	No	72.6±3.2	20.7±5.5	6.7±2.3
	Yes	86.6±2.6	22.3±2.3	4.1±0.3
MCF-7+SIgA	No	41.3±5.9*	45.9± 12.8*	12.8±2.8*
	Yes	82.9±4.5$^{\#}$	14.1±4.3$^{\#}$	4.9±0.3$^{\#}$
MCF-7+ PEG- SIgA	No	24.0±9.5*	70.5 ±12.5*	5.5±1.3$^{\#+}$
	Yes	80.0±3.1$^{\#}$	16.8±4.1$^{\#}$	3.1±1.1

Notes: The results represent the mean ± SD of five experiments. Polyethylene glycol (PEG) microspheres; secretory immunoglobulin A (SIgA); secretory immunoglobulin A adsorbed to PEG microspheres (PEG-SIgA). Q1: Viable cells (annexin$^-$/PI$^-$); Q2 (annexin$^+$/PI$^-$) and Q3 (annexin$^+$/PI$^+$): total apoptotic cells; Q4: necrotic cells (annexin$^-$/PI$^+$).*$p<0.05$ treated cells compared with non-treated cells (PBS). $^{\#}p<0.05$ indicates differences between MoAb anti-FcαR use within each treatment.

In general, the apoptosis index of MN cells was similar when these cells were incubated with PEG, SIgA, or SIgA adsorbed to PEG microspheres (Table 3). The apoptosis rate in co-cultures of MN cells and MCF-7 cells was higher. The co-culture of cells with SIgA increased the necrosis rates. The highest apoptosis index was observed in co-cultures of cells incubated with SIgA adsorbed to PEG microspheres (Table 3). In the presence of SIgA and PEG-SIgA, MN cells co-cultured with MCF-7 had lower apoptosis rates when pretreated with MoAb anti-FcαRI (Table 3).

Apoptosis is intensified in several diseases, including cancer [44, 45], and is essential in anticancer chemotherapy since it is promoted by the drugs used in this treatment [46-48]. Thus, the identifying substance that potentiates apoptosis in cancer cells is of great interest, especially when searching for new strategies for the prevention and treatment of breast cancer using natural proteins with potential anticancer properties [5], and their mechanisms of action are promising as a therapy for breast cancer [49].

Here, SIgA from the human colostrum adsorbed to PEG microspheres stimulated apoptosis in co-culture. Interestingly, adsorption of SIgA to the PEG microspheres reduced the necrosis, suggesting that the SIgA-PEG can modify the antibody's effects on cell death mechanisms both in tumor cells and in colostrum cells.

The effectiveness of PEG-SIgA depends on its binding to FcαRI. We indeed found that MN cells pretreated with MoAb anti-FcαR did not perform significant apoptosis rates in co-culture with MCF-7. FcαR binding can modulate the activation status of cells and consequently their immune responses. Neutrophils can kill IgA-opsonized tumor cells efficiently by strong induction of FcαRI signaling [50]. After binding FcαRI, IgA is internalized by MN cells [51] and probably participates in the specific immune responses against tumor cells.

These data confirm that a high concentration of phagocytes in the secretion and that the SIgA-cell interactions via its Fc alpha receptor (CD89 -6,12) probably are responsible for initiating different killing mechanisms [14] and induction of apoptosis [52].

4. Conclusions

Colostrum provides additional protection against breast cancer, seemingly through SIgA interactions with the Fcα receptors. The PEG-SIgA system's effects on colostral MN cells favoring chemokines' release with anti-tumor activity and apoptosis indices in co-culture with MCF-7 may be one of the mechanisms involved in protection against the breast tumor and a possible therapeutic alternative for treating this disease. Also, the interaction of these soluble and cellular components present in colostrum may be responsible for mechanisms that support the hypothesis that breastfeeding decreases the chance of women's breast cancer.

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Conflicts of Interest

Neither authors declare a conflict of interest and non-financial competing interests.

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