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Immunomodulatory Effects of Poly (Ethylene Glycol) Microspheres Adsorbed with Cortisol on Activity of Colostrum Phagocytes

Danny Laura Gomes Fagundes, Eduardo Luzía França,
Cristiane de Castro Pernet Hara and Adenilda Cristina Honorio-França
Institute of Biological and Health Science, Federal University of Mato Grosso,
Barra do Garças, MT, Brazil

Abstract: Cortisol is involved in a number of physiological and oxidative processes, including functional regulation in human milk. This hormone has been widely used for several therapeutic approaches. The aim of this study was to evaluate the immunomodulatory effect of Polyethylene Glycol (PEG) microspheres with adsorbed cortisol on the functional activity of colostrum phagocytes. The PEG microspheres were evaluated by flow cytometry and fluorescence microscopy were determined in colostrum phagocytes the effects of the PEG microspheres with adsorbed cortisol on the viability, superoxide release, phagocytosis, microbicidal activity and intracellular calcium release. The fluorescence microscopy and flow cytometry analyses revealed that the cortisol was able to adsorb PEG microspheres. The phagocytes display an increased release of superoxide in the presence of PEG microspheres with adsorbed cortisol. PEG microsphere adsorbed cortisol increased the phagocytosis and mediated bacterial killing by colostral mononuclear phagocytes. The cortisol adsorbed to PEG microspheres had direct effect on colostrum phagocytes and that they were able to increase the intracellular calcium release of colostrum phagocytes. These findings suggest that PEG microspheres with adsorbed cortisol present immunostimulatory effects in colostrum phagocytes and thus, these PEG microspheres have potential for future clinical applications in the infection process.

Key words: PEG microsphere, phagocytes, cortisol, colostrum

INTRODUCTION

Colostrum presents soluble immunological components and contains large amounts of viable leukocytes (1×10^9 cells mL^{-1} in the first days of lactation), especially phagocytes as neutrophils and macrophages (Goldblum and Goldman, 1994). The most cells present in colostrum are macrophages that represent the first line of defense of innate immune system. The soluble components of colostrum are able to interact with phagocytes and modulate the functional activity of these cells.

On the other hand the literature describes the importance of hormone as potent immunomodulator which participate in several aspects of the immune response (Franca *et al*., 2009a, b; 2010; Franca-Botelho *et al*., 2011). Interactions involving hormones and the activation of immunocompetent cells have been reported and suggest a mechanism of interaction between cells and the hormone which participate in several aspects of the immune response (Franca *et al*., 2009a; Franca-Botelho *et al*., 2011).

Cortisol, as a hormone present in colostrum, has been used to treat various diseases (Long *et al*., 2005; Reul and Chandramohan, 2007; Lim *et al*., 2007). Medications containing glucocorticoids are used in therapy for purposes including hormone replacement therapy, immunosuppressive therapy, anti-allergic and anti-inflammatory therapy. Cortisol shows activity in all types of inflammatory reactions, whether they are caused by pathogens, chemical or physical stimuli or an inappropriate immune response (Reul and Chandramohan, 2007).

Controlled drug release systems have been used to improve the pharmacological properties of drugs over systems with a conventional release. These systems include particulate carriers which are composed primarily of lipids and/or polymers that can alter the pharmacokinetics and biodistribution of the drugs with which they are associated or function as drug reservoirs (Allen, 2005).

PEG microspheres are polymeric particles that have the capacity to absorb organic compounds and that are considered a major drug carrier (Park *et al*., 2005). The

Corresponding Author: Eduardo Luzía França, Instituto de Ciências Biológicas e da Saúde, FMT, Barra do Garças, T, Rodovia BR 070, Km 05 s/nº, Barra do Garças, Brazil Tel: 55-66340121121 Fax: 55-6634021117

microspheres' adsorption capacity for organic compounds can be modified to improve their biological function (Scott *et al*., 2010). Synthetic polymers allow for controlled cell recognition and communication, triggering the modulation of the immune response, cell adhesion or signal transduction (Kiick, 2007) and they are important in diseases processes.

However, no studies have linked the cortisol hormone adsorbed to PEG microspheres to the immunomodulatory mechanisms. Colostrum is known to be a secretion with immunologically active components that act in an infant's intestine without causing inflammation and cortisol is an important hormone in the inflammatory process.

Interactions between cells and hormones associated with a delivery vehicle, such as PEG, may be able to modulate the immune system and trigger important processes in therapy. The objective of this study was to investigate the immunomodulatory effect of PEG microspheres with adsorbed cortisol on the functional activity of colostrum phagocytes.

MATERIALS AND METHODS

Subjects: Approximately 15 mL of colostrum was collected from clinically healthy women aged 18-35 years, after the volunteers had signed an informed consent form, at the Health System Program of Barra do Garças, Mato Grosso, Brazil (N = 30). All of the mothers had given birth to healthy term babies through vaginal delivery. Colostrum samples were collected in sterile plastic tubes between 48 and 72 h postpartum (Honorio-Franca *et al*., 1997; França *et al*., 2011a). All procedures were submitted for ethical evaluation and received institutional approval.

Poly ethylene glycol (PEG) microspheres preparation: The microspheres were obtained from PEG 6000 using a modification of the method described by Scott *et al*. (2010). Briefly, 20 g of PEG 6000 was suspended in 100 mL Phosphate-buffered Saline (PBS), diluted with a solution 2 g of sodium sulfate to PBS and incubated at 37°C for 45 min. After incubation, the PEG microspheres were diluted 3:1 in PBS and washed twice in PBS (500 xg, 5 min) and resuspended in PBS. The formation of microspheres was thermally induced by subjecting the solution to 95°C for 5 min. For adsorption, the suspensions of the PEG microspheres in PBS were incubated with the hormone cortisol (Sigma, St. Louis, USA -concentration 100 ng mL^{-1}) at 37°C for 30 min. The PEG microspheres with or without the hormone adsorbed were fluorescence labeled with a solution of Dylight-488 (Pierce Biotechnology, Rockford, USA - 10 μg mL^{-1}) overnight at room temperature in dimethylformamide at a 100:1 molar ratio of PEG:Dylight and analyzed by fluorescence microscopy.

Flow cytometry: Immunofluorescence staining with phycoerythrin (PE, Sigma, St. Louis, USA) was performed to assess the ability of PEG microspheres to bind with fluorescent markers compared with the microsphere poly(methyl methacrylate) (CaliBRITE - BD, San Jose, USA). The PEG microspheres were incubated with 5 μL of PE (0.1 mg mL^{-1}) for 30 min at 37°C. After the incubation, the PEG microspheres were washed twice in PBS containing BSA (5 mg mL^{-1}; 500 xg, 10 min, 4°C). In all experiments, the PEG microspheres were analyzed by flow cytometry. The study was performed on FACS Calibur (BD, San Jose, USA). The PEG microsphere size was compared with the sizes of BD microspheres (6-μm CaliBRITE 3 Beads, BD Cat. N°340486, San Jose, USA) those were bound or unbound to PE (Scherer *et al*., 2011). The ratio of the fluorescence intensity of PEG microsphere was expressed as the geometric mean fluorescence intensity and the size was calculated according to the geometric mean of a Forward Scatter (FSC).

Obtaining supernatant from human colostrum: Colostrum supernatant samples of different mothers were obtained by centrifugation (10 min, 160 xg, 4°C), the upper fat layer was discarded and the aqueous supernatant was stored at -70°C for later cortisol analyses.

Separation of colostral cells: About 15 mL of colostrum was collected from each woman in sterile plastic tubes. The samples were centrifuged (160 xg, 4°C) for 10 min which separated the colostrum into three different phases, a cell pellet, an intermediate aqueous phase and a lipid-containing supernatant, as described by Honorio-Franca *et al*. (1997). Cells were separated by a Ficoll-Paque gradient (Pharmacia, Upsala, Sweden), producing preparations with 98% pure mononuclear cells and were analyzed by light microscopy. Purified macrophages were resuspended independently in serum-free medium 199 at a final concentration of 2×10^{6} cells mL^{-1}.

Cortisol hormone dosage by an immunoenzymatic method: The cortisol concentration in the colostrum supernatant was determined using an ELISA kit from Kit

Accu Bind (IBL, Hamburg, Germany) (Ackermann *et al*., 2010). Reaction rates were measured by absorbance plate-reading spectrophotometer with a 405 nm filter. The results were calculated according to the standard curve and shown in μg dL^{-1}.

***E. coli* strain:** The enteropathogenic *Escherichia coli* (EPEC) used was isolated from the stools of an infant with acute diarrhea (serotype O111:H2, LA1, eae1, EAF1, bfp1). This material was prepared and adjusted to 10^7 bacteria mL^{-1}, as previously described (Honorio-Franca *et al*., 1997).

Release of superoxide anion: Superoxide release was determined by cytochrome C (Sigma, St. Louis, USA) reduction (Pick and Mizel, 1981; Honorio-Franca *et al*., 1997). Briefly, mononuclear phagocytes and bacteria were mixed and incubated for 30 min to allow phagocytosis. The cells were then resuspended in PBS containing 2.6 mM $CaCl_2$, 2 mM $MgCl_2$ and cytochrome C (Sigma, St. Louis, USA; 2 mg mL^{-1}). The suspensions (100 μL) were incubated for 60 min at 37°C on culture plates. The reaction rates were measured by their absorbance at 550 nm and the results were expressed as nmol O^{-2}. All of the experiments were performed in duplicate or triplicate.

Bactericidal assay: Phagocytosis and microbicidal activity were evaluated by the acridine orange method (Franca *et al*., 2011a). Equal volumes of bacteria and cell suspensions were mixed and incubated at 37°C for 30 min under continuous shaking. Phagocytosis was stopped by incubation on ice. To eliminate extracellular bacteria, the suspensions were centrifuged twice (160 xg, 10 min, 4°C). The cells were resuspended in serum-free 199 medium and centrifuged. The supernatant was discarded and the sediment was dyed with 200 μL of acridine orange (Sigma, St. Louis, USA; 14.4 g L^{-1}) for 1 min. The sediment was resuspended in cold 199 medium, washed twice and observed under an immunofluorescence microscope at 400 and 1000x magnification. The phagocytosis index was calculated by counting the number of cells ingesting at least 3 bacteria in a pool of 100 cells. To determine the bactericidal index, we stained the slides with acridine orange and counted 100 cells with phagocytized bacteria. The bactericidal index is calculated as the ratio between orange-stained (dead) and green-stained (alive) bacteria×100 (Franca *et al*., 2011a). All of the experiments were performed in duplicate or triplicate.

Immunofluorescence and flow cytometry: Immunofluorescence staining at the FACS Calibur (BD San Jose USA) to assess intracellular Ca^{2+} release in colostrum phagocytes was used. Cells were loaded with the fluorescent radiometric calcium indicator FLUO-3. Cell suspensions, pre-treated or not with cortisol (Sigma, final concentration of 10^{-7} M) were incubated at 37°C for 30 min under continuous stirring. Suspensions were centrifuged twice (160 xg, 10 min, 4°C) and resuspended in PBS containing BSA (5 mg mL^{-1}). This suspension was incubated with 5 μL of Fluo-3 (1 μg mL^{-1}) for 30 min at 37°C. After incubation, cells were washed twice in PBS containing BSA (5 mg mL^{-1}; 160 xg, 10 min, 4°C) and then analyzed by flow cytometry. Calibration and sensitivity were routinely checked using CaliBRITE 3 Beads (BD San Jose USA). Fluo-3 was detected at 530/30 nm filter for intracellular Ca^{2+}. The rate of intracellular Ca^{2+} release was expressed in geometric mean fluorescence intensity of Fluo-3. Data shown in the figures correspond to one of several trials performed.

Statistical analysis: An analysis of variance (ANOVA) was used to evaluate superoxide, phagocytosis and the bactericidal index in the presence or absence of PEG microspheres adsorbed with cortisol and intracellular calcium. Statistical significance was considered when $p<0.05$ (Zar, 1984).

RESULTS

Characterization of PEG microspheres: The fluorescence microscopy image (Fig. 1a) shows the PEG microspheres produced in PBS. This result confirms that our method produces different sizes of microspheres that are easily separated in the suspension. The microspheres produced retained their spherical structure without deformation (Fig. 1a). Fluorescence microscopy showed that PEG microspheres are able to absorb the cortisol and that the hormone is distributed throughout the surface of the PEG microsphere (Fig. 1b).

Figure 2a compares the PEG microsphere, the PEG microsphere adsorbed with cortisol and the BD microsphere (standard) in terms of fluorescence intensity. The PEG microsphere and BD microsphere had highest geometric mean fluorescence intensity. The adsorption of cortisol to the PEG microsphere did not modify the geometric mean fluorescence intensity. When comparing size, the PEG microsphere was similar to the standard microsphere (Fig. 2b, c).

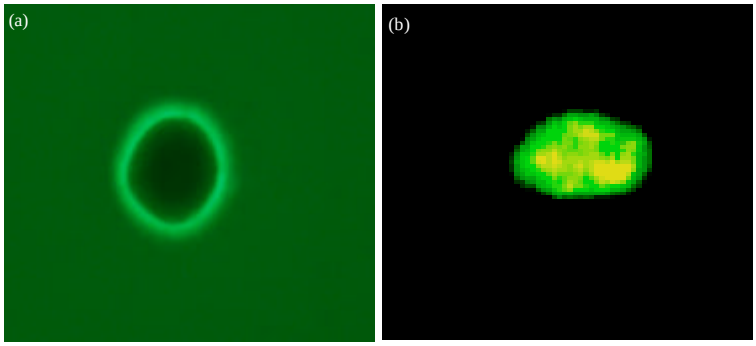
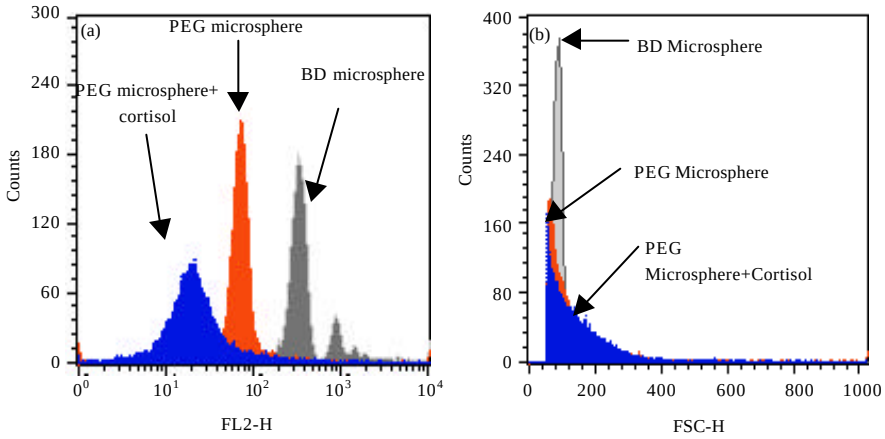


Fig. 1(a-b): Fluorescence microscopy image of the polyethylene glycol (PEG) microspheres stained with Dylight-488 (100x - panels - A), (a) PEG microsphere and (b) PEG microsphere adsorbed to cortisol hormone, Experiments were repeated five times and the results were comparable



(c) Microspheres	Size (μm)	Fluorescence intensity (%; Mean±SD)
Poly(methyl methacrylate) (BD)	6	83.1±5.1
Polyethylene glycol (PEG)	5.8	65.3±4.7
Polyethylene glycol (PEG) +Cortisol	5.9	69.2±5.6

Fig. 2(a-c): PEG microspheres were stained directly with phycoerythrin (PE) (a) Poly(methyl methacrylate) PE-labeled microspheres (BD Microsphere - Becton Dickinson, San Jose, USA) were used as standard. Immunofluorescence analysis and size were then carried out by flow cytometry (FACScalibur, Becton Dickinson, San Jose, USA). Fluorescence intensity (FL2), (b) size according to a Forward Scatter (FSC) and (c) Geometric mean fluorescence intensity and the geometric mean of size

General characteristic of colostrum components: The colostrum phagocytes count retrieved was 2.8×10^{6} cell mL^{-1} and the viability (%) was 90.5±2.2. Colostral cortisol concentration was 11. 9±1.3 μg dL^{-1}.

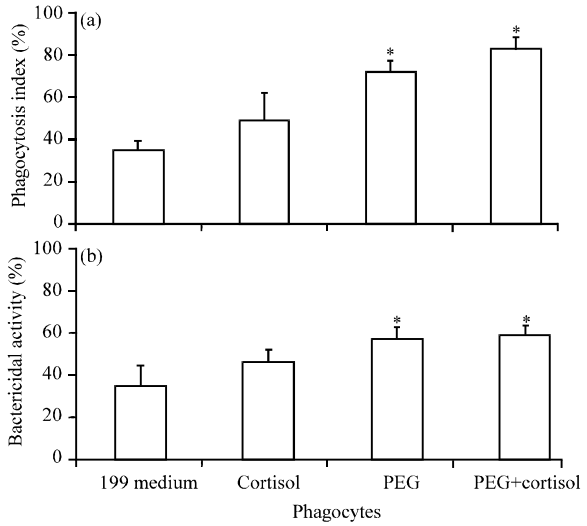


Fig. 3(a-b): (a) Bacterial phagocytosis and (b) Microbicidal activity (b) by colostrum mononuclear cells (Mean±SD, N = 10 in each treatment). Bacterial phagocytosis and elimination index by mononuclear cells from colostrum was determined with the acridine orange method. * indicates differences between treatment polyethylene glycol-PEG or/and hormone and 199 medium (ANOVA, $p<0.05$)

Table 1: Superoxide release by colostrum mononuclear (MN) cells

Colostrum MN phagocytes	Superoxide release (nmol)
Control (without bacteria)	0.7±0.3
Bacteria+PBS	1.7±0.2*
Bacteria+Cortisol	1.4±0.1*
Bacteria+PEG microsphere	1.6±0.4*
Bacteria+PEG microsphere with cortisol adsorbed	2.7±0.5*+

Mean±SD, N = 10 in each treatment, the mononuclear cells were incubated with cortisol. In the controls assays, the mononuclear cells were pre-incubated with Phosphate-Buffered Saline (PBS). *$p<0.05$ comparing the treated cells with cells non-treated (without bacteria), *$p<0.05$ comparing the different treatment (PBS, cortisol and polyethylene glycol-PEG microsphere

The effect of PEG microspheres adsorbed with cortisol on superoxide release: The cortisol increased superoxide release by colostrum phagocytes compared with their spontaneous release (spontaneous = 0.7±0.3-cortisol 1.4±0.1; Table 1). The bacteria stimulated superoxide release (1.7±0.2). Phagocytes incubated with bacteria and cortisol had higher superoxide release (spontaneous = 0.7±0.3-bacteria plus cortisol = 1.4±0.1). Additionally, in the presence of cortisol adsorbed to PEG microspheres, the phagocytes display an increased release of superoxide compared with phagocytes exposed to the PEG microsphere only (bacteria plus PEG microsphere = 1.6±0.4-bacteria plus PEG microsphere plus cortisol = 2.7±0.5) and release was higher than with cortisol stimulation (bacteria plus cortisol = 1.4±0.1; Table 1).

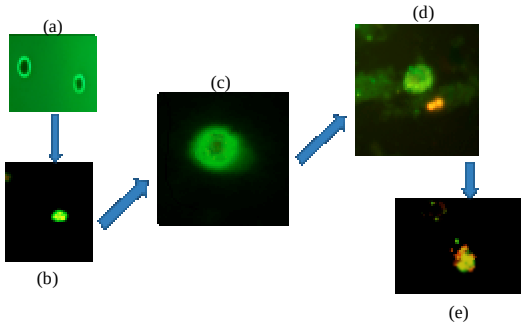


Fig. 4(a-e): Bacterial killing by colostral mononuclear phagocytes, (a) PEG microsphere, (b) Cortisol adsorbed to PEG microspheres, (c) Mononuclear phagocytes with cortisol adsorbed to PEG microspheres internalized. The mononuclear phagocytes were incubated with bacteria on a shaker for 30 min at 37°C. After washing at 4°C, cells were stained with acridine orange and analyzed by fluorescent microscopy. Orange-stained bacteria (dead) and green- stained bacteria (alive), (d) Phagocytosis of bacteria and (e)bacterial killing

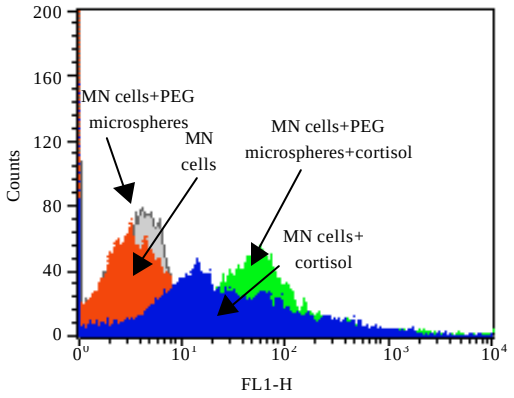


Fig. 5: Colostrum mononuclear cells (MN) stimulated with cortisol adsorbed to PEG microsphere were staining with Fluo-3 to assess intracellular Ca^{2+} release as described in Materials and Methods. Immunofluorescence analyses were then carried out by flow cytometry (FACScalibur, Becton Dickinson, San Jose, USA)

The effects of PEG microspheres adsorbed with cortisol on the phagocytosis of colostrum mononuclear cells: Colostral phagocytes displayed some phagocytic activity in response to EPEC (34.4±4.6). Phagocytosis increased significantly in the presence of cortisol (48.8±1.8). A

Table 2: Intracellular Ca^{2+} release by mononuclear (MN) colostrum cells in the presence of cortisol adsorbed to PEG microsphere indicated by fluorescence intensity

Colostrum MN cells	Intensity of fluorescence (% Mean±SD)
PBS	13.9±2.1
Cortisol	19.8±0.7*
PEG microsphere	21.2±1.2*
PEG microsphere with cortisol adsorbed	25.6±7.1*+

The results represent the mean and SD of five experiments with cells of different individuals. *$p<0.05$ comparing the treated cells with cells non-treated (phosphate-buffered saline-PBS). +$p<0.05$ comparing the different treatment (Cortisol and Polyethylene Glycol-PEG microsphere)

comparison of the PEG microspheres adsorbed with cortisol with PEG microspheres alone showed that phagocytosis, in general, was similar (PEG microsphere = 72.0±5.1-PEG microsphere plus cortisol = 82.0±6.2; Fig. 3).

The effects of PEG microspheres adsorbed with cortisol on the bactericidal activity of colostrum phagocytes: In general, colostrum mononuclear phagocytes that were not stimulated had some bactericidal activity against EPEC (34.9±9.5). Mononuclear phagocytes incubated with cortisol showed similar bactericidal activity to phagocytes that were not stimulated (199 medium = 34.9±9.5-cortisol = 45.9±5.8). Mononuclear phagocytes incubated with cortisol adsorbed to the PEG microspheres showed increased microbicidal activity in response to EPEC (58.7±4.7; Fig. 3). Colostral mononuclear phagocytes were able to internalize bacteria, irrespective of the use of cortisol adsorbed to PEG microsphere. Cortisol adsorbed to PEG microsphere mediated bacterial killing by colostral mononuclear phagocytes is shown in Fig. 4.

Effect of PEG microspheres adsorbed with cortisol on the intracellular Ca^{2+} release of colostrum phagocytes: The incubation of colostrum phagocytes with cortisol revealed that the cells had increased intracellular Ca^{2+} levels (Fig. 5). Table 2 shows the rate of intracellular Ca^{2+} release using Fluo-3 to assess the fluorescence intensity of colostral phagocytes treated with PEG microspheres adsorbed with cortisol. The highest intracellular Ca^{2+} release were found in MN phagocytes treated with PEG microspheres adsorbed with cortisol (Table 2).

DISCUSSION

The major findings of the present study was that PEG microspheres with adsorbed cortisol had immunostimulatory effects in human colostrum phagocytes showed by effects on superoxide release, bacterial-killing activity and intracellular Ca^{2+} release.

Microsphere-based polymeric substances can be employed as release delivery systems for drugs and therapeutic proteins. In this study, was a used microparticles of PEG as a potential carrier system for hormones, because PEG has biocompatible characteristics and its degradation products do not present toxicity and are easily metabolized and excreted by normal physiological pathways (Henning, 2002). PEG formulations are characterized by presenting a prolonged half-life, reduced side effects and ultimately increased therapeutic efficiency (Davis, 2002).

In this study, an analysis by fluorescence microscopy and flow cytometry showed that the PEG microspheres were of regular size, easily separated from the suspension and able to adsorb cortisol and interact with colostrum phagocytes to modulate their functional activity. The literature has reported the use of flow cytometry as an alternative method for the analyses and visualization of particles (Stadler *et al*., 2011). Here, showed by flow cytometry that the PEG microsphere has a size of about 5.8 μm and that it was able to adsorb cortisol on its surface without changing its size or ability to bind to fluorescent substances. The PEG microspheres are a type of copolymer that are used in the clinical administration of drugs because of their incorporation capacity (Novelli *et al*., 1993), their ability to increase the duration of drug exposure or other products such as enzymes and their role as an important signaling vehicle in immunity (Wang *et al*., 2009). The rate of drug release is controlled by two factors and it is important to understand the physical and chemical properties of the releasing medium (Freiberg and Zhu, 2004). By modulating the size of the pores and the drug concentration, PEG microsphere formulations may allow controlling the speed by which the drug is released from the polymer matrix (Zanettil *et al*., 2002).

Several drugs associated with PEG that are able to extend the time of the relative bioavailability of the drug when compared with the free drug and to potentiate the pharmacological action, such as alpha interferon (PEGasys®, PEG-Intron),® growth factor hormone (Somavert®), asparaginase (Oncaspar®) and insulin, are widely sold (Jevsevar *et al*., 2010). In this study, the PEG microspheres with adsorbed cortisol showed that this system can be an important vehicle to enhance immunological processes.

In the literature, there are reports that the hormone cortisol has been used therapeutically in the treatment of immune disorders, inflammatory and acute diseases (Rady *et al*., 2006) and that the endogenous release of this hormone can directly modulate immune function and play a central role in the immune response (Kohut *et al*., 2005). In vitro assays revealed that at low concentrations, glucocorticoids have an effect on the immunostimulatory

activity of macrophages, whereas at high concentrations, they have an immunosuppressive effect (Long *et al*., 2005; Lim *et al*., 2007).

The elucidate the therapeutic concentrations of cortisol and the characteristics of PEG, in this study, we used nanodoses of this hormone to improve the therapeutic processes and regulatory functions of phagocytes in colostrum, because this secretion is easy to obtain, is non-invasive and does not pose a risk for either the mother or child. Moreover, these phagocytes act locally without triggering inflammatory processes.

On the other hand, human colostrum plays an important role in protecting the mucosa of the newborn, because it has significant amounts of cells and other soluble components (Carneiro-Sampaio *et al*., 1996; Franca *et al*., 2011a, b). In the present study, we found that the colostrum presents cortisol levels in values similar to those observed in human serum (Watts and Tindall, 1988).

Studies have shown that blood and the mammary gland are responsible for the secretion of hormones important for the development and maturation of the gastrointestinal tract and immune system of newborns (Bernt and Walker, 2009; Sauter *et al*., 2004). These hormones temporarily regulate the activity of some endocrine glands until the hormonal system of the newborn is fully developed (Bernt and Walker, 2009). Therefore, the hormones secreted in milk are considered necessary for the health and growth of newborns and are important modulators of immune functions.

In this study, the cortisol adsorbed to PEG microsphere was able to increase the release of superoxide anions by colostrum phagocytes. Under oxidative stress, cells are able to generate large amounts of superoxide radicals (Franca *et al*., 2009a,b, 2011a). The generation of free radicals has been reported as an important defense mechanism of organisms during infectious processes, especially in intestinal infections (Honorio-Franca *et al*., 1997; Franca-Botelho *et al*., 2011; Franca *et al*., 2011a).

On the other hand, PEG is a polymer that has properties important for the modulation and prolongation of drug action. Thus, the adsorption of cortisol in the PEG microspheres suggested to be an important immunostimulatory cellular oxidative process and efficient for infection control, as this hormone in large doses has an immunosuppressive effect (Lim *et al*., 2007). The results of this study confirm the importance of the superoxide anion for bacterial activity and drug delivery systems. The increase in superoxide release by PEG microspheres adsorbed with cortisol affects the phagocytic and bactericidal activities of colostrum cells.

Phagocytosis and microbicidal activity by both blood and colostral phagocytes, with substantial participation of active oxygen metabolites such as free radicals (Honorio-Franca *et al*., 1997; Ferrari *et al*., 2009), have been considered an important defense mechanism that protects infants against several bacterial (Honorio-Franca *et al*., 1997; Franca *et al*., 2011a) and protozoan infections (Franca-Botelho *et al*., 2011).

The literature reports that soluble components present in colostrum interact with cells and increase superoxide release and that this increase is directly related to phagocytic and bactericidal activity (Long *et al*., 2005; Franca *et al*., 2011a). This work is the first to report the increased functional activity of colostrum phagocytes associated with hormones in a controlled release system. The microbicidal activity and the products derived from oxidative metabolism promoted by cortisol may have important clinical implications. Increased superoxide modifies the response of intracellular Ca^{2+} and phosphorylation events during oxidative metabolism (Carrichon *et al*., 2011). This study shows that the PEG microspheres adsorbed to cortisol have direct effects on colostrum phagocytes and that they are able to increase the intracellular calcium release of colostrum phagocytes. Cortisol has been reported to increase intracellular calcium in lymphoblasts irrespective of phosphatidyl inositol (Gardner and Zhang, 1999) and this action may be linked to the intracellular action of this hormone. These results suggest that the increase of superoxide by phagocytes in the colostrum in the presence of PEG microspheres adsorbed with cortisol can alter the levels of intracellular Ca^{2+} and promote the microbicidal activity of these cells. The interaction of hormones associated with controlled release systems and colostrum cells may be of fundamental importance for newborns, especially those who need milk substitutes. Because of the immaturity of the digestive function of the neonate, the cells received through colostrum are not destroyed by digestive enzymes and other factors and are likely to remain intact in the upper portions of the intestine; thus, they can interact with other components and protect the mucosa. Studies have suggested that colostrum cells remain viable in the intestinal mucosa for a period of 4 h (Hugaes *et al*., 1998; Caspari, 1993) and those they may have microbicidal activity in the newborn.

The functional activity of colostrum phagocytes mediated by PEG microspheres with adsorbed cortisol may represent an additional mechanism for the innate protection and treatment of gastrointestinal infections in newborns during the first days of life. The functional activity of phagocytes modulated by the hormone cortisol can likely be extended to other pathogens.

The complex relationship between the immune system and anti-inflammatory or infectious processes indicates the importance of glucocorticoids in therapy for a variety of diseases and new mechanisms for modulating PEG microspheres with adsorbed cortisol are necessary to develop new nanomaterial treatments, especially considering the cost-effectiveness of combination therapy drug delivery systems.

CONCLUSION

In this study indicates that PEG microspheres with adsorbed cortisol have immunostimulatory effects in human colostrum phagocytes and may represent a possible therapy alternative for children with infections or immunosuppression.

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