Food and Chemical Toxicology 50 (2012) 4310–4315

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

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

Lactobacillus reuteri CRL 1098 and *Lactobacillus acidophilus* CRL 1014 differently reduce *in vitro* immunotoxic effect induced by Ochratoxin A

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ARTICLE INFO

Article history:
Received 3 April 2012
Accepted 16 July 2012
Available online 5 September 2012

Keywords:
Ochratoxin A
Lactobacilli
Cytokines
Apoptosis
Membrane rafts

ABSTRACT

Ochratoxin A (OTA) is a widespread mycotoxin contaminating several food products which causes detrimental health effects. The ability of *Lactobacillus reuteri* CRL 1098 and *Lactobacillus acidophilus* CRL 1014 to prevent OTA effects on TNF-α and IL-10 production and apoptosis induction in human peripheral blood mononuclear cells (PBMC) was investigated. Membrane rafts participation in these responses was also evaluated. *L. reuteri* reduced by 29% the OTA inhibition of TNF-α production whereas *L. acidophilus* increased 8 times the TNF-α production by OTA treated-PBMC. Also, both bacteria reversed apoptosis induced by OTA by 32%. However, neither of the bacteria reversed the OTA inhibition on IL-10 production. On the other hand, the lactobacilli were less effective to reverse OTA effects on disrupted-rafts PBMC. This study shows that two lactobacilli strains can reduce some negative OTA effects, being membrane rafts integrity necessary to obtain better results. Also, the results highlight the potential capacity of some lactobacilli strains usually included in natural dietary components in milk-derived products and cereals feed, to reduce OTA toxicity once ingested by humans or animals.

1. Introduction

Ochratoxin A (OTA) is a small organic toxin of 403.8 Da produced as a secondary metabolite by many of the common fungal molds such as *Aspergillus* and *Penicillium* species. It is a widespread mycotoxin contaminating several food products, such as cereals, coffee, dried fruits, beer and animal derived products (Scudamore et al., 2003; Suarez-Quiroz et al., 2005). Most cereals, grains and other feed derivatives are susceptible to fungal growth (Magnoli et al., 2005). *Aspergillus* and *Penicillium* can grow on grain stored at 15–19% humidity and temperatures higher than 15 °C (Petzinger and Ziegler, 2000). Frequently, most factors contributing to the presence or production of OTA in food or feed, including storage, environmental conditions such as temperature and humidity, and ecological conditions, are beyond human control (Hussein and Brasel, 2001) and for these reasons the prevention of OTA contamination of food is very difficult.

OTA toxic effects include hepatotoxicity, nephrotoxicity and immunotoxicity (Petzinger and Ziegler, 2000). OTA has been shown to be immunosuppressive *in vivo* and *in vitro* (Müller et al., 1999; Stormer and Lea, 1995). Studies in mice have shown that OTA treatment induced depletion of lymphoid cells and suppression of the antibody response (Müller et al., 1995). OTA also downregulates lymphocyte proliferation in cells of murine and human origins (Stormer and Lea, 1995; Thuvander et al., 1995) and damages neutrophils in a dose dependent manner, impairing the immune function of leukocytes and cytokines production (Odhav et al., 2008).

Lactic acid bacteria (LAB) are normal habitants of the gastrointestinal tract from humans and animals (Shu et al., 1999). *Lactobacillus* and *Bifidobacterium* species are considered beneficial to the host and as such have been used as probiotic, defined as "live microorganisms which when administered in adequate amounts confer health benefits to the host" (FAO/WHO, 2002). The beneficial properties of LAB as food protective agents are highly appreciated and they have been widely studied. Previously, LAB protective effects against bacterial toxins produced by *Clostridium difficile* were demonstrated (Trejo et al., 2010). This report suggests that co-cultures of *C. difficile* with probiotic *Lactobacillus* or *Bifidobacterium* strains modify the microenvironment of *C. difficile*, which modifies signaling pathways associated with toxin production. Likewise, LAB protective effects against mycotoxins have been previously reported. Two probiotic strains, *Lactobacillus rhamnosus* GG

Abbreviations: DMSO, dimethyl sulfoxide; ELISA, Enzyme Linked Immunosorbent Assay; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; IL-10, Interleukin 10; LAB, lactic acid bacteria; MRS, de Man Rogosa and Sharpe; MβCD, methyl-β-cyclodextrin; OTA, Ochratoxin A; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; TNF-α, Tumor Necrosis Factor alpha.

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http://dx.doi.org/10.1016/j.fct.2012.07.070

and *L. rhamnosus* LC-705, are capable to bind and remove the mycotoxin Aflatoxin B1 (El-Nezami et al., 1998; Gratz et al., 2006). Regarding OTA, it was published that some wine LAB are capable of removing by adsorption this mycotoxin, reducing its toxicity (Del Prete et al., 2007). It has been widely demonstrated that probiotics beneficial properties are mainly related to their role as immune modulators of pro- and anti-inflammatory cytokines balance (Chon and Choi, 2010; Elmadfa et al., 2010; Hong et al., 2009). However, reports concerning probiotics effects against OTA immunotoxicity are not available. OTA immunotoxicity has been investigated in multiple species, including rodents, poultry and pigs (Bondy and Pestka, 2000). Among the possible mechanisms participating in the immunosuppression induced by OTA, an induction of apoptosis of human lymphocytes has been reported (Assaf et al., 2004).

Lactobacillus strains are capable of interacting with immune cells and we demonstrated that some lactobacilli strains modulate TNF-α production by peripheral blood mononuclear cells (PBMC), effect that was dependent on the integrity of membrane rafts (Soria et al., 2008). These microdomains (rich in cholesterol, glycosphingolipids and glycosylphosphatidylinositol anchored proteins) concentrate a wide range of receptors, thus acting as target platforms for microorganisms (bacteria, viruses, parasites) and bacterial toxins (Riethmüller et al., 2006). Several toxins interact with membrane rafts, and membrane raft disruption impairs their toxicity (Lin et al., 2011; Nagahama et al., 2004).

This study was undertaken to analyze *in vitro* the effect of two probiotic lactobacilli strains on OTA immune-toxicity by using the peripheral blood mononuclear cells (PBMC) model described by Katial et al. (1998). TNF-α and IL-10 production and apoptosis were evaluated as parameters of cellular response. Also, the role of membrane rafts in OTA toxic effect was studied.

2. Materials and methods

2.1. Microorganisms and growth conditions

Lactobacillus (*L.*) *reuteri* CRL 1098 and *Lactobacillus acidophilus* CRL 1014 were obtained from the Culture Collection of the Centro de Referencia para Lactobacilos (CERELA, Tucumán, Argentina). The strains were grown in MRS (de Man, Rogosa and Sharpe medium, Britania, Buenos Aires, Argentina) at 37 °C (OD_{560} = 0.50). The lactobacilli cells were harvested by centrifugation, washed with phosphate-buffered saline (PBS) and resuspended in RPMI 1640 medium (GIBCO, Grand Island, NY, USA) for co-cultures assays. The microorganisms viability was determined by colony-forming unit (CFU) counting on MRS agar after 48 h incubation at 37 °C.

2.2. PBMC isolation and membrane rafts disruption

Peripheral blood mononuclear cells were isolated by density gradient centrifugation with Ficoll-Hypaque (Histopaque-1077 Hybri-Max, Sigma, St. Louis, MO, USA) from heparinized whole blood from healthy donors (range 24–42 years old). The isolated PBMC were washed twice with PBS and resuspended in RPMI 1640 medium. To disrupt membrane rafts, PBMC were treated with 10 mM methyl-β-cyclodextrin (MβCD, Sigma, St. Louis, MO, USA) in RPMI 1640 medium at 37 °C for 10 min (Soria et al., 2008). Control and disrupted-rafts PBMC were adjusted to 2×10^6 cells ml^{-1}. The cells were incubated in 24-well plates (TPP, Switzerland) for 4–24 h at 37 °C with or without 10% (v/v) fetal bovine serum (Natocor, Córdoba, Argentina) in presence or absence of *L. reuteri* CRL 1098 (4×10^7 CFU ml^{-1}) and *L. acidophilus* CRL 1014 (2×10^7 CFU ml^{-1}). PBMC viability was determined by the Trypan Blue (Sigma, St. Louis, MO, USA) exclusion assay. In every experiment, the cellular viability was higher than 95%.

2.3. MTT assay

5×10^4 PBMC were incubated with OTA 5, 7.5, 10, 20, 50 and 100 μg ml^{-1} in a 96-well plate (TPP, Switzerland) at 37 °C under 5% CO_2 for 4 and 8 h. Cytotoxicty was measured using the CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega, MA, USA). After washing the plate, 100 μl of RPMI 1640 medium were added with 15 μl of dye solution to each well and incubated at 37 °C and 5% CO_2 for 4 h. Then, 100 μl of solubilization/stop solution were added to each well and incubated at 37 °C overnight.

2.4. OTA assays

Ochratoxin A (OTA, Sigma, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA) to prepare a 50 mM stock solution. PBMC were incubated at 37 °C under 5% CO_2 for 4–24 h with different OTA concentrations (5–100 μg ml^{-1}). A control for DMSO was included in each assay.

2.5. Cytokines quantification

TNF-α and IL-10 production in control and disrupted-rafts PBMC, incubated in presence or absence of the bacteria was measured in the co-culture supernatants by Enzyme Linked Immunosorbent Assay (ELISA, eBioscience, San Diego, CA, USA), performed according to the manufacturer's instructions.

2.6. Apoptosis measurements

Apoptosis of control and disrupted-rafts PBMC was detected by flow cytometry using a FITC Annexin V Apoptosis Detection Kit I (Becton Dickinson, San Jose, CA, USA). Briefly, PBMC were harvested by centrifugation, washed with cold PBS and resuspended in 1X Binding Buffer at a final concentration of 1×10^6 cells ml^{-1}. Five micro liters Annexin V and Propidium Iodide (PI) were added to 100 μl of the cell suspension (1×10^5 cells) and incubated 15 min at room temperature in dark. 1X Binding Buffer (400 μl) was added to each sample before flow cytometry analysis. Unstained and Annexin V or PI stained PBMC were used as controls of labeling. The percentage of viable and apoptotic cells was analyzed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) using the FCS Express V3 software.

2.7. Statistical analysis

All assays were carried out in triplicate and the results were expressed as mean values with standard deviations. Statistical analysis was performed using the MINITAB 14 software (State College, PA, USA). Comparisons were accomplished by ANOVA general linear model followed by Tukey's post hoc test. A p value <0.05 was considered statistically significant (*) and ($^\#$).

3. Results

The cytotoxic effect of OTA on PBMC was assessed by MTT assay. PBMC were exposed to different OTA concentrations (5, 7.5, 10, 20, 50 and 100 μg ml^{-1}) from 4 to 8 h. Neither of these OTA concentrations showed cytotoxic activity on PBMC up to 8 h of incubation (data not shown). Considering these results, the modifications induced by OTA on two cytokines (TNF-α and IL-10) production were evaluated, using these non-cytotoxic OTA concentrations. OTA had a harmful effect on both TNF-α and IL-10 production by PBMC in a dose- and incubation time-dependent manner (Fig. 1). For the lowest OTA concentration tested (5 μg ml^{-1}), no differences were observed regarding TNF-α production by PBMC in comparison with OTA non-treated cells, up to 8 h of incubation. For 7.5 μg ml^{-1} OTA, TNF-α production by PBMC was reduced by 68% and 52% after 4 and 8 h of incubation, respectively. For both OTA concentrations (5 and 7.5 μg ml^{-1}), a complete inhibition in TNF-α production was observed after 24 h (Fig. 1A). For 10 μg ml^{-1} OTA, TNF-α production was markedly reduced (more than 90% inhibition) and for higher concentrations (20, 50 and 100 μg ml^{-1}) TNF-α production was undetectable (data not shown).

Regarding IL-10, a decreased production of the cytokine was observed with OTA treatment at all dose and incubation times evaluated (Fig. 1B). However, the lowest reduction of IL-10 production was obtained after treatment with 7.5 μg ml^{-1} OTA for 4 h, with a reduction percentage of 84% compared with PBMC without OTA treatment (Fig. 1B).

Considering that 5 μg ml^{-1} OTA produced slight modifications on TNF-α production and 10 μg ml^{-1} or higher OTA concentrations strongly reduced the cytokines production, 7.5 μg ml^{-1} OTA and 4 h incubation time were selected as the experimental conditions for further assays with the lactobacilli strains. The effects of lactobacilli strains on OTA inhibition of cytokines production by PBMC are shown in Fig. 2. *L. reuteri* partially reversed (29%) the inhibition of TNF-α production induced by OTA (Fig. 2A). On the other hand, *L. acidophilus* not only completely reversed the inhibitory OTA effect

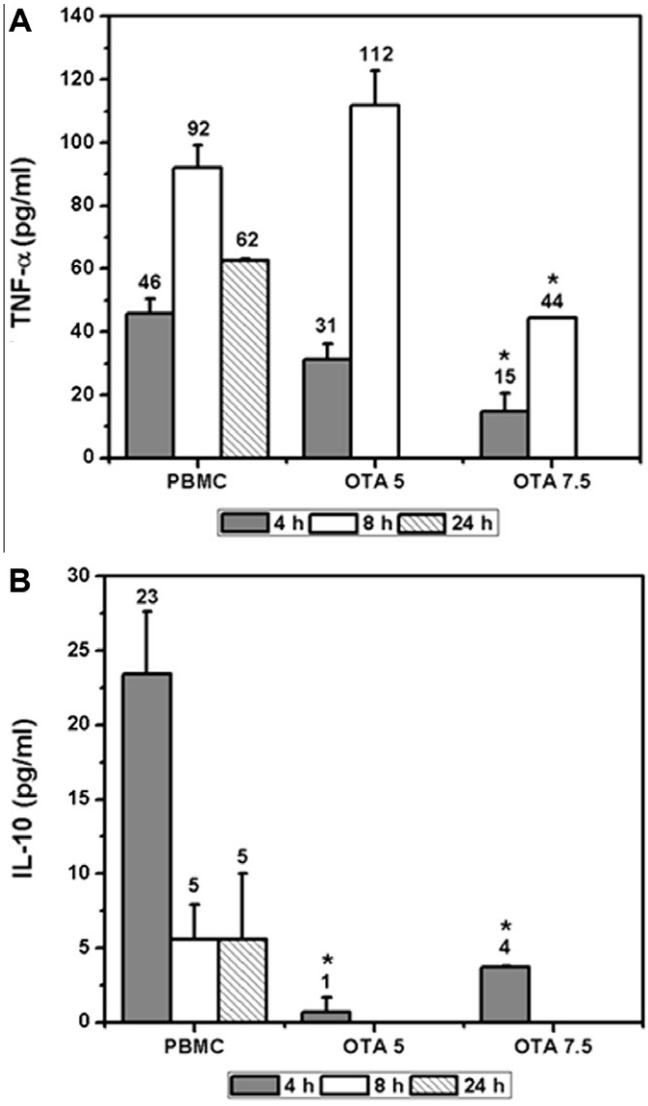

Fig. 1. Effect of OTA on cytokine production by PBMC. PBMC were incubated with different OTA concentrations for 4 to 24 h at 37 °C. (A) TNF-α and (B) IL-10 production was measured in culture supernatants by ELISA. Statistical significance $^{*}p < 0.05$ as compared with control cells. Each value represents the mean ± SD obtained from at least three different experiments.

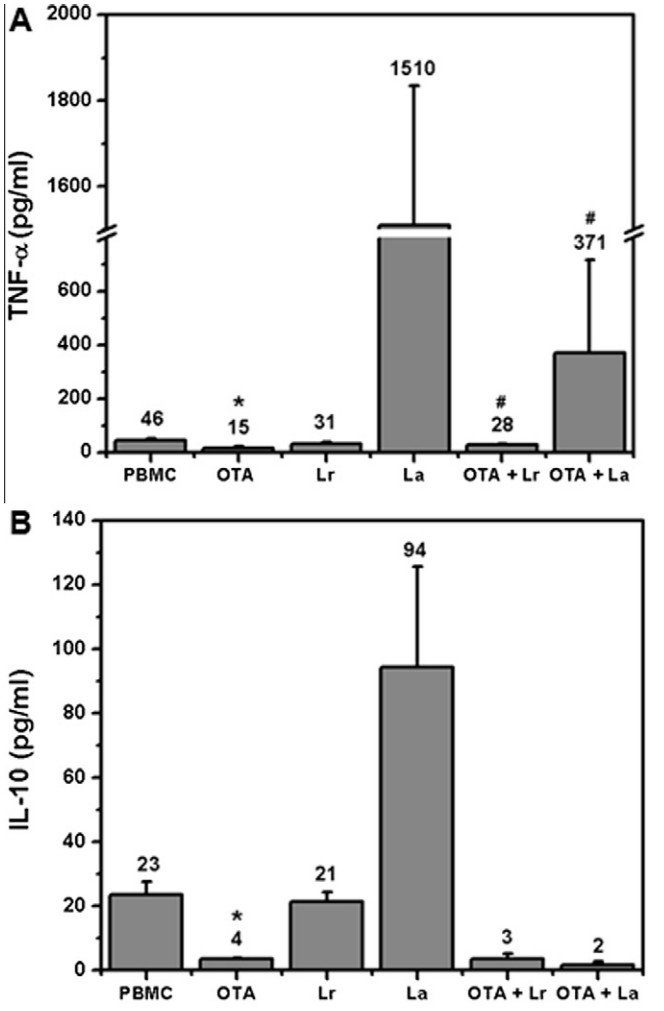

Fig. 2. Effect of *L. reuteri* and *L. acidophilus* on cytokine production by OTA in PBMC. PBMC were treated with 7.5 $\mu g ml^{-1}$ OTA and incubated with *L. reuteri* (Lr) and *L. acidophilus* (La) to evaluate their effect on (A) TNF-α and (B) IL-10 production. Statistical significance $^{*}p < 0.05$ as compared with PBMC and $^{\#}$as compared with OTA-treated PBMC. Each value represents the mean ± SD obtained from at least three different experiments.

on TNF-α production but also, despite OTA inhibition, induced an increase of 8 times (808%) in the TNF-α production by PBMC (Fig. 2A). In contrast, the lactobacilli strains were not able to reverse the OTA effect on IL-10 production by PBMC (Fig. 2B). Regarding apoptosis of PBMC, OTA presence markedly increased the percentage of apoptotic PBMC from 7% to 20% (Fig. 3). However, the addition of the lactobacilli strains to the OTA cultured cells reduced apoptosis by 32% (Fig. 3).

To investigate whether lipid rafts play a role in OTA (deleterious) and lactobacilli (beneficial) effects, the cytokines production and apoptosis in disrupted-rafts PBMC (dr-PBMC) were determined. OTA exerted a 90% inhibition of TNF-α production in dr-PBMC (Fig. 4), a higher effect in comparison with control PBMC (68%, Fig. 1A). *L. reuteri* had only a slight effect on the reversal of TNF-α production by OTA-treated dr-PBMC: 17% (Fig. 4A). On the other hand, *L. acidophilus* increased by 30% the TNF-α production in OTA-treated dr-PBMC (Fig. 4A). In dr-PBMC, the effectiveness of the lactobacilli strains to reverse OTA inhibition on TNF-α production was lower than in non-disrupted-rafts cells (see Fig. 2A). Likewise, as in non-disrupted-rafts PBMC, the lactobacilli strains were not able to reverse the OTA inhibition on IL-10 production by dr-PBMC (Fig. 4B). Apoptosis percentage in dr-PBMC was similar to non-disrupted rafts PBMC (Fig. 5) and the addition of *L. reuteri* and *L. acidophilus* decreased OTA-induced-apoptosis by 21% and 15%, respectively (Fig. 5).

4. Discussion

OTA is produced in a wide variety of climates and geographical regions (O'Brien and Dietrich, 2005). Almost all types of food can be contaminated, so human exposure has been demonstrated worldwide and its origin seems to be the intake of contaminated food. OTA effects on human health have been demonstrated to be dependent on the level of exposure to the toxin and the persistence of the exposure. Furthermore, the risk of human exposition to OTA is associated to the level of food contamination and the geographical areas, since OTA contamination varies according to them. The occurrence of OTA in food varies from each type of product, country and sometimes for each city. For example, values of 5.4 and 3.7 $\mu g g^{-1}$ were detected in rye flour and wheat flour, respectively, in Poland (Golinski et al., 1991). OTA in wheat reached a

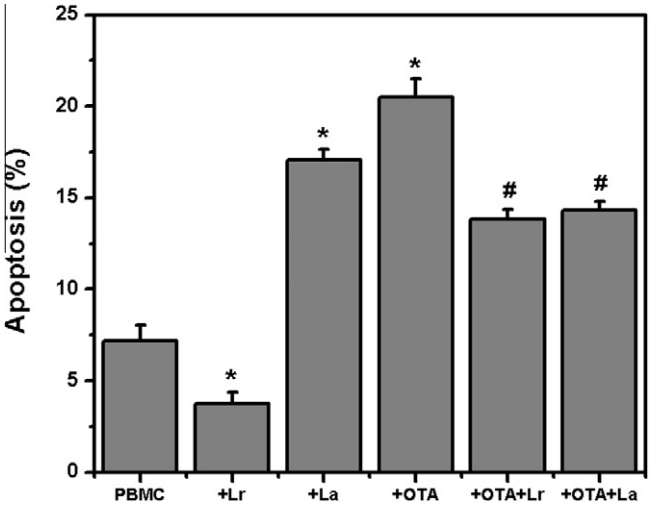

Fig. 3. Effect of *L. reuteri* and *L. acidophilus* on apoptosis induced by OTA in PBMC. PBMC were incubated with 7.5 μg ml^{-1} OTA for 4 h at 37 °C. *L. reuteri* (Lr) or *L. acidophilus* (La) were incubated with PBMC to evaluate their effect on apoptosis induced by OTA. Statistical significance $^{*}p < 0.05$ as compared with PBMC and $^{\#}$as compared with OTA-treated PBMC. Each value represents the mean ± SD obtained from at least three different experiments.

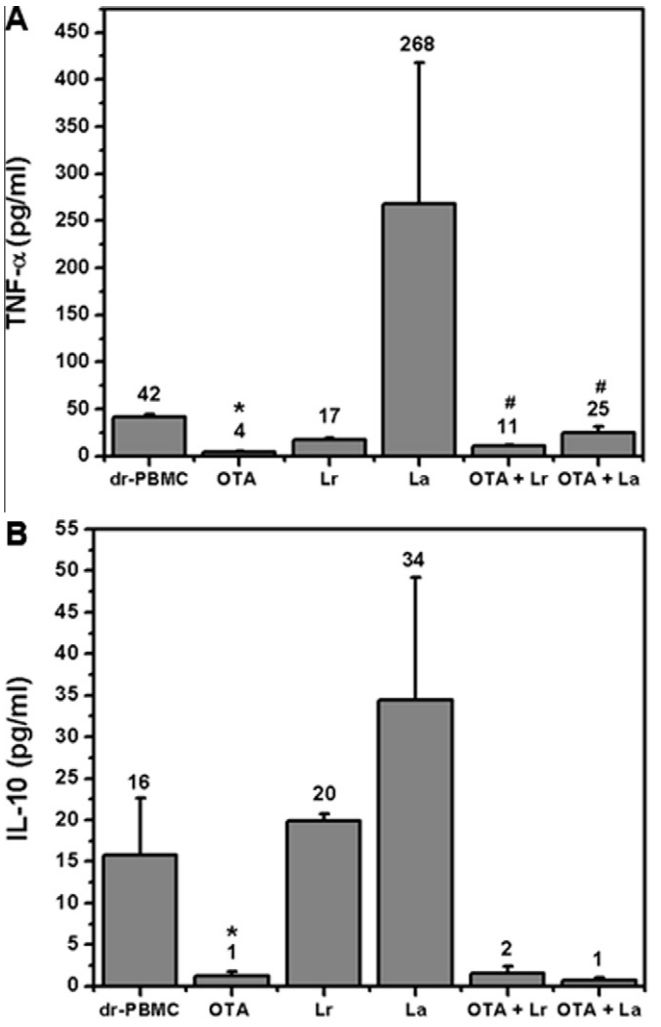

Fig. 4. Effect of *L. reuteri* CRL 1098 and *L. acidophilus* CRL 1014 on cytokine production in disrupted-rafts PBMC (dr-PBMC) incubated with OTA. dr-PBMC were treated for 4 h at 37 °C with 7.5 μg ml^{-1} OTA and incubated with: *L. reuteri* (Lr) and *L. acidophilus* (La) to evaluate their effect on (A) TNF-α and (B) IL-10 production. Statistical significance $^{*}p < 0.05$ as compared with dr-PBMC and $^{\#}$as compared with OTA-treated dr-PBMC. Each value represents the mean ± SD obtained from at least three different experiments.

value of 2.4 μg g^{-1} in Poland, while the value detected in Denmark was not higher than 0.037 μg g^{-1} (Rizzo et al., 2002).

Previous risk assessments focused their attention on genotoxicity and carcinogenicity, and ignored the limited and outdated immunotoxicity reports (Al-Anati and Petzinger, 2006). The available data on OTA immunotoxicity are difficult to understand and often contradictory (Foligne et al., 2007). It is important to note that OTA is frequently found in the human blood due to its widespread contamination in food and grains (Mally et al., 2007). More important, OTA is a persistent toxin with a blood half-life of thirty-five days following a single oral dose, which renders the toxin one of the most frequently retained mycotoxin in human circulation (Petzinger and Ziegler, 2000). OTA levels in human circulation is the result of pharmacokinetics parameters (body absorption, excretion, etc.) which also determine OTA in other organs (Coronel et al., 2010). In humans, mean OTA plasma concentrations resulting from dietary intake within the European Union are at least three orders of magnitude lower (0.18–1.19 ng ml^{-1}, corresponding to 0.45–2.95 nM) (SCOOP, 2002). However, in some countries such as Tunisia, exceptionally high concentrations of up to 1,136 ng ml^{-1} (2.8 μM) have been reported in some individuals suffering from kidney or urinary disorders (Maaroufi et al., 1995). Even when the link between dietary OTA exposure and human diseases has not been determined, it seems reasonable to assume that cellular response observed in *in vitro* studies may also occur in individuals exposed to extremely high levels of OTA.

The aim of this study was to test the OTA immunotoxic effect using an *in vitro* cellular model of peripheral blood mononuclear cell and to evaluate the effectiveness of two *Lactobacillus* strains to reduce OTA effects. The parameters tested were cytokines production (TNF-α and IL-10) and apoptosis. The selection of OTA doses assayed was supported by other *in vitro* studies with human lymphopcytes, which employed an OTA dose ranging from 20 to 100 μg ml^{-1} (Odhav et al., 2008) and 5 to 10 μM (2 and 4 μg ml^{-1}, respectively) (Assaf et al., 2004), as well as one with immortalized human kidney epithelial (IHKE) cells incubated with OTA at concentrations between 0 and 100 μM (Rached et al., 2006). Even though the concentrations used in our study are higher than detected in human plasma (Studer-Rohr et al., 2000), the MTT assays demonstrated neither of the OTA concentrations assayed in the present study (from 1 to 100 μg ml^{-1}) showed cytotoxic activity on PBMC up to 8 h of incubation (data not shown). Additionally, a previous study using an *in vivo* rat model detected OTA in plasma in a range from 5.4 to 42.8 μg ml^{-1} depending on the dose and method used to administered the toxin (up to 1 mg ml^{-1}, administered either orally or by gavage) (Miljkovic et al., 2003). Results from the present work showed that OTA induced TNF-α and IL-10 inhibition in PBMC by a dose- and incubation time-dependent manner. Modifications on cytokines production, such as IL-10, IL-1 and IL-6, induced by this mycotoxin in lymphocytes and neutrophils were previously reported (Odhav et al., 2008). Regarding TNF-α inhibition, other mycotoxin, citrinin, showed this effect in alveolar epithelial cells (Johannessen et al., 2007). However, the mechanism by which OTA modifies cytokine production is not fully assessed. Nevertheless, inhibition of protein synthesis by OTA is thought to be responsible for most of their toxic effects, indirectly impairing the activity of cellular enzymes involved in cytokines production (Ringot et al., 2006). In our experimental model, the most important while not excessively suppressive effect on cytokines production exerted by OTA was obtained using 7.5 μg ml^{-1}. Thus, this concentration was selected for further assays using LAB strains.

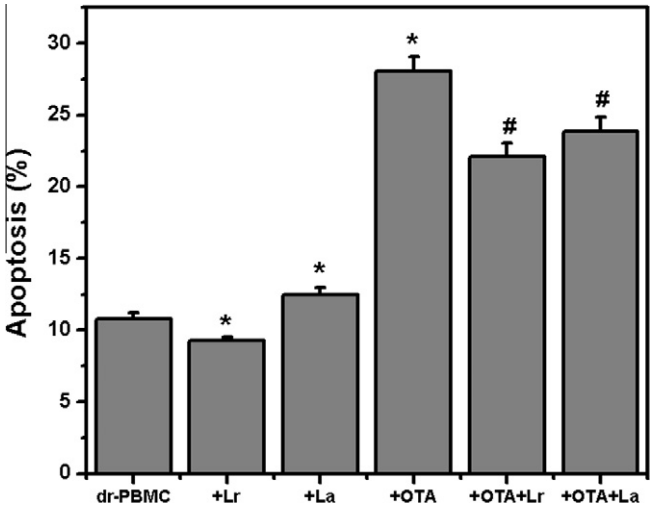

Fig. 5. Effect of *L. reuteri* CRL 1098 and *L. acidophilus* CRL 1014 on apoptosis in disrupted-rafts PBMC (dr-PBMC) incubated with OTA. dr-PBMC were incubated with 7.5 μg ml^{-1} OTA for 4 h at 37 °C. *L. reuteri* (Lr) or *L. acidophilus* (La) were incubated with dr-PBMC to evaluate their effect on apoptosis induced by OTA. Statistical significance $^{*}p < 0.05$ as compared with dr-PBMC and $^{\#}$as compared with OTA-treated dr-PBMC. Each value represents the mean ± SD obtained from at least three different experiments.

To date, several compartments of the immune system have been identified as affected by oral probiotic delivery, including lymphocyte function and innate cell defenses (Cross, 2002). Although the first target for immune response induction is at the gut intestinal level, there is evidence that the immunomodulatory effects of probiotics can be expressed systemically. In fact, stimulated lymphocyte in the lymphoid tissue associated to the gut can migrate to other distant location, and can eventually be detected in peripheral blood. That is why PBMC cultures are commonly used as an *in vitro* model of mature and functional lymphocytes (Castellazzi et al., 2007; Dong et al., 2010; Haller et al., 2000; Pérez-Cano et al., 2010). Moreover, it has been reported that microbial component and cells driven from normal microflora can cross the gut epithelial layer and interact with the immune cells even from distant locations (Clarke et al., 2010; Ishibashi and Yamazaki, 2001). While contact between gut-dwelling bacteria and intestinal epithelial cells may be considered part of the routine microbial signaling processes of a healthy gut microflora, it has been shown that epithelial cells cultures are limited to perform complex immune response and our work tried to focus on the immunotoxicity of OTA. *In vitro* studies with the CaCo-2 human intestinal epithelial cell line have shown that probiotic *Lactobacillus johnsonii* can induce the expression of the anti-inflammatory mediator TGF-β, but not pro-inflammatory cytokines, such as TNF-α or IL-1β. Addition of leukocytes to CaCo-2 *Lactobacillus* cocultures promotes the production of pro-inflammatory molecules by the epithelial cells, but also induces secretion of the leukocyte-derived anti-inflammatory IL-10 (Haller et al., 2000).

In this study, *L. reuteri* CRL 1098 and *L. acidophilus* CRL 1014 were tested on their capacity to reverse some toxic effects observed in blood cells *in vitro*. OTA detrimental effects on immune system have been investigated in animal *in vivo* models and in cellular *in vitro* models (Bondy and Pestka, 2000). The two strains studied in our work showed different capacities to modify cytokine inhibition induced by OTA. None of the bacteria were able to reverse IL-10 inhibition induced by OTA. However, *L. reuteri* CRL 1098 partially reversed TNF-α inhibition induced by OTA, whereas *L. acidophilus* CRL 1014 strongly diminished the toxic effect on this cytokine production. The different protective capacities of *L. reuteri* and *L. acidophilus* against OTA immunosuppressive effect, indicate that the immunomodulatory effect of LAB is strains dependent. These findings are consistent with previous results obtained in the murine monocyte/macrophage cell line J774A.1, where two *Lactobacillus* strains were able to modify the production of several cytokines in a different manner (Cross et al., 2004).

Induction of apoptosis in PBMC is another toxic OTA effect investigated in this study. OTA induces apoptosis in different cell types, such as primary rat hepatocytes and MDCK-C7 cells (Chopra et al., 2010). OTA induced apoptosis in PBMC, but apoptosis induction was minor in presence of lactobacilli strains: both, *L. reuteri* and *L. acidophilus*, diminished apoptosis in PBMC exposed to OTA. In OTA untreated cells, *L. reuteri* produces anti-apoptotic effects and *L. acidophilus* pro-apoptotic effects. The different effect exerted in OTA-treated cells could be explained, in the case of *L. reuteri*, by the inhibition of TNF-α production, a pro-apoptotic cytokine. As *L. acidophilus* increased TNF-α production, their anti-apoptotic effect in PBMC treated with OTA could be due to another mechanism of apoptosis, independent on TNF-α signaling. The results reported in our study suggest that each bacterium could trigger different mechanisms to diminish apoptosis induced by OTA, as an alteration of the balance between pro- and anti-inflammatory cytokines or by a mitochondrial pathway (Chiu et al., 2010). The ability of cells to undergo apoptosis is very important to eliminate cells which no longer should be proliferating, such as cells experiencing mutagenic changes. Since OTA is mutagenic, the capacity of the *Lactobacillus* strains to reduce OTA mutagenicity also needs to be tested. Since *L. reuteri* CRL 1098 and *L. acidophilus* CRL 1014 were able to reverse the OTA toxic effects assayed in the present work, it might be possible that these lactobacilli strains are also capable of reducing the mutagenic changes induced by OTA and, thereby, the anti-apoptotic activity exerted by them would result in a beneficial property.

The membranes of eukaryotic cells possess specialized microdomains, termed as membrane rafts, which may be the preferential interaction site between a variety of toxins and bacteria with the target cells (Fivaz et al., 2000). Membrane rafts disruption was reported to be responsible for some toxic effects of mycotoxins in mammalian cells lines (Brandwagt et al., 2000). The results obtained in this study with dr-PBMC indicate that membrane rafts could be involved in the mechanism of reduction of cytokine production (TNF-α and IL-10) and apoptosis induced by OTA. *Lactobacillus* strains used in this study require the membrane rafts integrity for modulation of TNF-α production by PBMC (Soria et al., 2008). In dr-PBMC incubated with OTA, the reversal of TNF-α production exerted by *L. reuteri* was similar to that observed in PBMC without raft modifications. Moreover, while *L. acidophilus* strongly reversed the toxic effect on TNF-α production in OTA-treated PBMC, this effect was achieved in less extent in disrupted-rafts PBMC. The marked differences in the capacity of *L. reuteri* and *L. acidophilus* to reverse OTA effects in dr-PBMC indicate that membrane rafts integrity may be involved in this response.

5. Conclusion

In summary, the results provide clues that support the capacity of some LAB for restricting some toxic effects of OTA like cytokines depletion and apoptosis. The use of these bacteria as natural dietary components in some milk or cereal feed, could restrict OTA toxicity once ingested by humans or animals. Further studies will be required to provide information concerning the mechanisms by which LAB reduce OTA immunotoxicity, and the potential role of membrane rafts in this effect. This work is currently underway in our laboratory.

Conflict of Interest

The authors declare that there are not conflicts of interest.

Acknowledgements

This work was supported by Grants from CONICET, Agencia Nacional de Promoción Científica y Tecnológica, Argentina and CIUNT-UNT.

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