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Probiotic *Lactobacillus* strains protect against myelosuppression and immunosuppression in cyclophosphamide-treated mice

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ABSTRACT

This work evaluated the capacity of two probiotic strains, *Lactobacillus casei* CRL431 and *Lactobacillus rhamnosus* CRL1506, to protect against myelosuppression and immunosuppression in cyclophosphamide (Cy)-treated mice. Changes in mature granulocytes and progenitor cells in bone marrow (BM) and blood were studied. In addition, the ability of probiotics to accelerate the recovery of the immune response against the opportunistic pathogen *Candida albicans* was evaluated. We demonstrated for the first time that the preventive treatment with immunomodulatory lactobacilli such as *L. casei* CRL431 or *L. rhamnosus* CRL1506 was able to increase immature myeloid progenitors in the BM, allowing an early recovery of myeloid cells after Cy administration. Probiotic lactobacilli were also capable to induce an early recovery of neutrophils in blood, improve phagocytic cells recruitment to infectious sites and increase the resistance against the opportunistic pathogen *C. albicans*. Although deeper studies regarding the cellular and molecular mechanisms of probiotic actions are needed, these findings support the idea that strains like CRL431 and CRL1506 may accelerate the recovery of Cy-caused immunosuppression by immunopotentiating myeloid cells. Then, probiotic lactobacilli have the potential to be used as alternatives for lessening chemotherapy-induced immunosuppression in cancer patients.

1. Introduction

Cyclophosphamide (Cy) is one of the most widely used alkylating agent and a major constituent of combined chemotherapy regimens [1]. Cy has a high cytotoxicity on tumor cells and therefore it is used in the treatment of acute and chronic leukemias [2,3], multiple myeloma [4], lymphoma [5,6], autoimmune diseases [7] and patient preparation for bone marrow transplant [1,8]. However, Cy has low specificity and it has a broad spectrum of cytotoxic effects on normal cells [9]. Among the side effects caused by Cy highlights the induction of myelosuppression, since this drug interferes with the proliferation and differentiation of cells in the bone marrow (BM) [10–12]. These changes in BM are associated to a marked leukopenia and neutropenia [13,14] and to an increase in the susceptibility to infections [15,16].

Management of immunocompromised patients under chemotherapy treatments is complex and infectious diseases are among the most important problems in this population. In these patients, the occurrence of bacterial and fungal infections is usually very serious and can be rapidly fatal if untreated [17,18]. Early hospitalization and intravenous treatment with colony stimulating factors and broad-spectrum antibiotics have achieved a significant reduction in the number of infections in these patients. However, there are obvious problems resulting from hospitalization as well as the toxicity of these drugs, psychological and economic decline. For these reasons, it is essential to avoid damage of non-malignant cells during the clinical application of Cy, in order to reduce morbidity and mortality of infections in immunocompromised patients.

Many attempts are being investigated to find safe immune-potentiating agents able to reduce myelosuppression and improve immune response in chemotherapy-treated patients. In this regard, scientists have emphasized the importance of functional foods and dietary supplements for health promotion [19]. The use of probiotic foods for improving immune health status in immunocompromised patients has gained a special interest in recent years [20]. In this sense, our laboratory and others conducted studies in different experimental models of immunosuppression in order to evaluate the capacity of probiotic lactic acid bacteria (LAB) to improve immunity. The results of these investigations showed that some LAB strains are able to improve the resistance against various pathogens such as *Pseudomonas aeruginosa, Gardnerella vaginalis, Streptococcus pneumoniae, Candida albicans* and *Salmonella*

Abbreviations: BM, bone marrow; CFU, colony forming unit; Cy, cyclophosphamide; DCs, dendritic cells; LAB, lactic acid bacteria; Lc431, *Lactobacillus casei* CRL431; Lr1506, *Lactobacillus rhamnosus* CRL1506; MPO, myeloperoxidase; PBS, phosphate buffer saline; Px+, peroxidase positive.

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aeruginosa in immunocompromised hosts [21]. We demonstrated in an experimental model of malnourished immunocompromised mice that a repletion diet supplemented with *Lactobacillus casei* CRL431 or *L. rhamnosus* CRL1506 accelerated the normalization of innate and specific immune responses against pneumococcal infection [22–24]. Moreover, taking into account the relationships between the hematopoietic and the immune systems, and the immunomodulatory effect of these probiotic LAB strains, we also demonstrated that the addition of probiotic microorganisms to repletion diets improved innate and adaptive immunity in immunocompromised hosts and that this effect is mediated in part by a beneficial influence on hematopoiesis [22,23,25,26]. These results provide the scientific basis for proposing probiotic LAB strains as potential adjuvant to minimize the deleterious effects associated with antineoplastic therapy, especially those related to myelotoxicity and immunosuppression.

Therefore, the aim of this work was to evaluate the capacity of *L. casei* CRL431 and *L. rhamnosus* CRL1506 to protect against myelosuppression and immunosuppression in Cy-treated mice. For this purpose, we determined the daily changes in mature granulocytes and progenitor cells in blood and BM after chemotherapy. Furthermore, we measured the effect of preventive treatments with probiotic LAB on blood leucocytes recovery as well as progenitor and stem cell mobilization in the BM. In addition, we evaluated the ability of probiotics to accelerate the recovery of the immune response against the opportunistic pathogen *Candida albicans*.

2. Materials and methods

2.1. Probiotic microorganisms

Lactobacillus casei CRL431 and *L. rhamnosus* CRL1506 were obtained from the CERELA culture collection. Lactic acid bacteria (LAB) (stored at −70 °C) were activated and cultured for 18 h at 37 °C (final log phase) in Man–Rogosa–Sharpe broth. The microorganisms were harvested by centrifugation and washed three times with sterile 0.01 mol/l phosphate buffer saline (PBS), pH 7.2. Finally, bacteria were suspended in 10% non-fat milk to be administered to mice. Both strains were selected from several LAB strains in preliminary experiments in Cy-treated mice (data not shown).

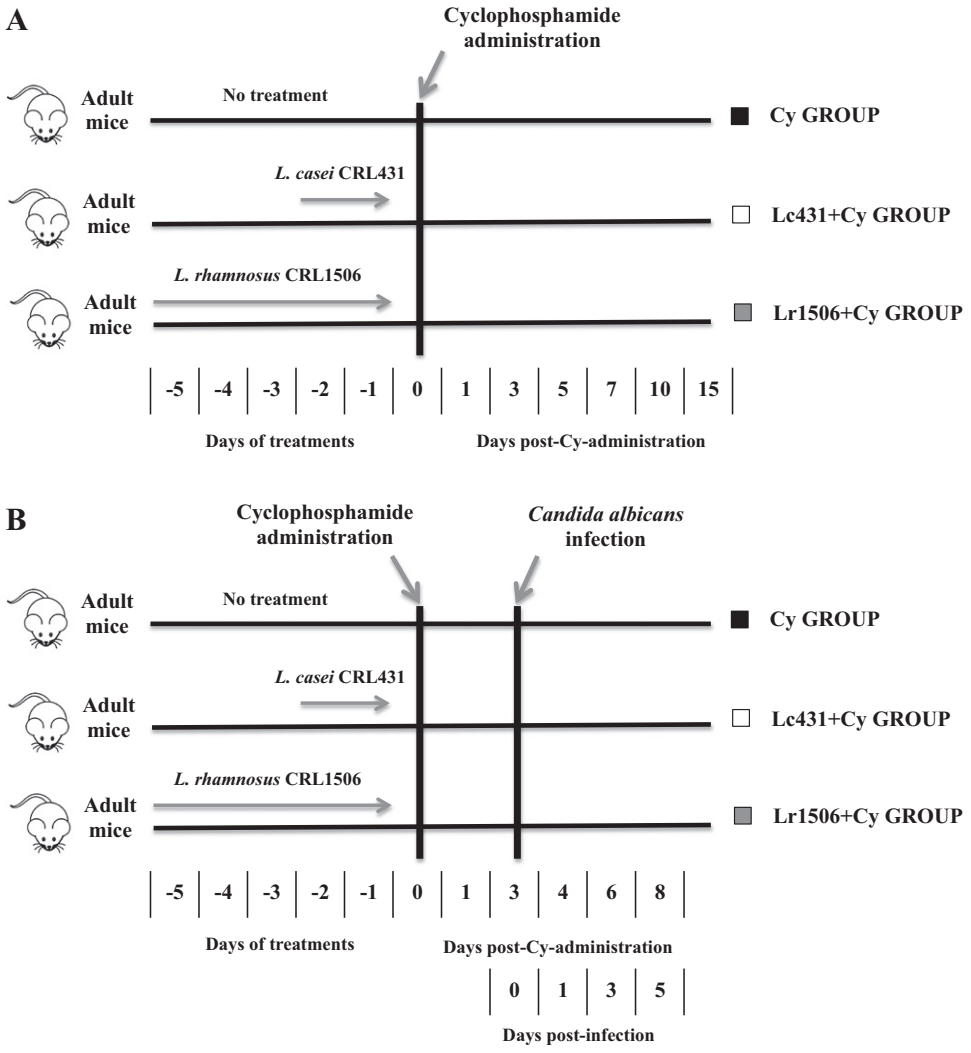

Fig. 1. Experimental protocols used in this work. (A) Male 6-week-old Swiss mice were fed with *Lactobacillus casei* CRL431 (Lc431) or *Lactobacillus rhamnosus* CRL1506 (Lr1506) for 2 consecutive days at a dose of 10^9 cells/mouse/day or 5 consecutive days at a dose of 10^8 cells/mouse/day respectively. The treated groups and the untreated control mice received one dose cyclophosphamide (Cy, 150 mg/kg) intraperitoneally. Determinations were performed on day 0 (before Cy administration) and in different time points after Cy administration, during 15 days. Six animals per each time point per groups were used in the experiments. (B) Male 6-week-old Swiss mice were fed Lc431 or Lr1506 as described before. The treated groups and the untreated control mice received one dose of Cy (150 mg/kg) intraperitoneally. Challenge with *Candida albicans* (intraperitoneal injection of 10^7 cells) was performed on the day 3 after Cy administration, which was considered day 0. Determinations were performed on day 0 (before infection) and on days 1, 3 and 5 post-infection (dpi). Six animals per each time point per groups were used in the experiments.