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Beneficial immunomodulatory activity of *Lactobacillus casei* in malnourished mice pneumonia: effect on inflammation and coagulation

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Abstract

Objective: The effect of *Lactobacillus casei* CRL 431 immunomodulatory activity on inflammation and coagulation during pneumococcal pneumonia was investigated in malnourished mice.

Methods: Weaned mice were malnourished after they consumed a protein-free diet for 21 d. Malnourished mice were treated for 7 d with a balanced conventional diet (BCD) with *L. casei* supplementation (BCD+Lc) or without it. The malnourished control group received only a protein-free diet whereas well-nourished control (WNC) mice consumed BCD ad libitum. Mice were challenged by the intranasal route with pneumococci at the end of each dietary treatment. Lung injury, leukocyte recruitment, cytokine production, coagulation tests, and fibrin(ogen) deposition in lungs were evaluated.

Results: Malnourished control mice showed impaired leukocyte recruitment and cytokine production, and more severe lung injuries when compared with WNC mice. Coagulation tests were significantly impaired in malnourished control group versus WNC group. Repletion with BCD or BCD+Lc improved these parameters, but only BCD+Lc mice achieved the values of WNC mice. In addition, the interleukin-10 level was higher in the BCD+Lc group than in the WNC group.

Conclusion: Repletion with supplemental *L. casei* accelerated recovery of the defense mechanisms against pneumococci by inducing different cytokine profiles. These cytokines would be involved in the improvement of the immune response and in the induction of a more efficient regulation of the inflammatory process, limiting the injury caused by infection.

Keywords: *Lactobacillus casei*; Malnourished mice; Inflammation; Coagulation system; Pneumococcal pneumonia

Life-threatening complications from bacterial infections are a major and growing clinical problem, aggravated by the emergence and spread of antibiotic resistance in bacterial pathogens and by an increase in the number of immunocompromised patients [1].

During bacterial infection, the host responds to invading microbes with a number of different defense mechanisms. Blood coagulation and inflammation are universal responses to infection and there is a cross-talk between them that can either amplify or dampen their respective functions. Loss of appropriate interactions between these systems contributes to tissue damage in infectious diseases [2].

Bacterial pneumonia is a leading cause of morbidity and mortality and *Streptococcus pneumoniae* remains the most common pathogen responsible for community-acquired pneumonia in both developed and developing countries [3]. Pneumococcal pneumonia is characterized by an intense inflammatory reaction that is known to be directly induced by pneumococcal cell wall components and pneumolysin [4]. In addition, there is growing evidence that aspects of the immune response greatly contribute in pneumococcal pathogenesis: although immunosuppressed individuals die as a consequence of poor host response, immunocompetent hosts face overwhelming inflammatory reactions that contribute to tissue injury, shock, and death [5].

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Modern therapeutic approaches to infectious diseases focus on the modulation of the host response, especially in immunocompromised individuals [6]. In this sense, the use of probiotics to beneficially modulate the immune system has greatly increased in recent years. Probiotic lactic acid bacteria has several immunomodulatory effects, documented in different studies by various research groups, which include improvement of the immune response [7–10] and antiinflammatory properties [11–13].

In a previous study we demonstrated in a pneumonia-sepsis model [7] that malnourished mice are more susceptible to *S. pneumoniae* infection. In addition, we reported that repletion of malnourished mice with a balanced conventional diet (BCD) with supplemental *Lactobacillus casei* CRL 431 (BCD+Lc) significantly accelerates the recovery of the defense mechanisms against pneumococcal respiratory infection.

The effect of protein malnutrition on the interaction between inflammation-coagulation has not been studied extensively. Moreover, there are no reports concerning the potential effect of probiotic lactic acid bacteria in this situation. In this work we undertook a series of experiments to investigate the effect of *L. casei* immunomodulatory activity on inflammation and coagulation in malnourished mice challenged with a dose of *S. pneumoniae* that induces pneumonia without sepsis.

Materials and methods

Microorganisms

L. casei CRL 431 was obtained from our culture collection (Chacabuco 145, San Miguel de Tucumán, Argentina). The culture was kept freeze-dried and then rehydrated using the following medium: peptone 15.0 g, tryptone 10.0 g, meat extract 5.0 g, and distilled water 1 L, pH 7. It was cultured for 8 h at 37°C (final log phase) in Man-Rogosa-Sharpe broth (Oxoid, Wesel, Germany). The bacteria were harvested by centrifugation at 3000 × g for 10 min and washed three times with sterile 0.01 mol/L phosphate-buffered saline (PBS), pH 7.2. Capsulated pneumococcus (serotype 14) was isolated from the respiratory tract of a patient from the Department of Clinical Bacteriology of the Niño Jesús Children's Hospital in San Miguel de Tucumán, Argentina. Pneumococci serotypification was performed in Administración Nacional de Laboratorios e Institutos de Salud, Buenos Aires, Argentina.

Animal model

Male 6-wk-old Swiss albino mice were obtained from the closed colony kept at our bioterio. They were housed in plastic cages at room temperature. Mice were housed individually during the experiments, and the assays for each parameter studied were performed in 5 to 6 mice per group for each time point. Weaned mice were malnourished after they consumed a protein-free diet [7] for 21 d. At the end of this period, mice that weighed 45% to 50% less than well-nourished mice were selected for experiments. Well-nourished control (WNC) mice consumed ad libitum a BCD [7].

Malnourished mice were separated into two groups for repletion treatment. One group of malnourished mice was fed BCD for 7 consecutive days. Because administration of *L. casei* for 2 d is the optimal dose to provide protection against *S. pneumoniae* in malnourished mice [7], the second group of mice received 7 d of BCD+Lc with *L. casei* supplementation (10^9 colony-forming unit/mouse/d) on Days 6 and 7. The malnourished control (MNC) group received only the protein-free diet whereas WNC mice consumed the BCD ad libitum. Our ethical committee for animal care approved the experiments.

Experimental infection

S. pneumoniae was first grown on blood agar for 18 h; freshly grown colonies were suspended in Todd Hewitt broth (Oxoid) and incubated at 37°C overnight. The pathogen was harvested through centrifugation at 3000 × g for 10 min at 4°C and then washed three times with sterile PBS. Cell density was adjusted to 4×10^4 colony-forming unit/L. The size of the inoculum was confirmed by serial dilutions and quantitative subcultures on blood agar. Challenge with *S. pneumoniae* was performed on the day after the end of each dietary treatment (Day 8). Mice were infected by dropping 25 μL of the inoculum containing 10^3 log-phase colony-forming unit of *S. pneumoniae* in PBS into each nostril and allowing it to be inhaled. To facilitate migration of the inoculum to the alveoli, mice were held in a head-up vertical position for 2 min. WNC and MNC mice were infected in the same way. Mice were sacrificed on Day 0 (before infection) and at 12 h and 1, 2, 3, 5, 10, and 15 d postinfection. The infecting dose was selected on the basis of its inability to produce translocation to blood, as determined from the results of the bacterial cell counts performed in the blood of mice with severe pneumonia (unpublished data). During the 15 d postinfection all groups were fed only with BCD, with the exception of MNC group, which received protein-free diet.

Determination of leukocytes in bronchoalveolar lavages and biochemical assay of bronchoalveolar lavage fluid

Bronchoalveolar lavage (BAL) samples were obtained according to the technique previously described [7]; briefly, the trachea was exposed and intubated with a catheter and two sequential BALs were performed in each mouse by injecting 0.5 mL of sterile PBS. A portion of the fluid was used to determine the total number of leukocytes using a hemocytometer. The remaining sample of fluid was centrifuged for 10 min at 900 × g, the pellet was used to make smears, and differential cell counts were performed by