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Lactobacillus casei DN-114 001 inhibits the increase in paracellular permeability of enteropathogenic *Escherichia coli*-infected T84 cells

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Abstract

Probiotics are living microorganisms which, when ingested in adequate amounts, exert health benefits toward the host. For instance, probiotics might act through reinforcement of the intestinal epithelial barrier function. The goal of the present study was to determine whether *Lactobacillus casei* DN-114 001 could abrogate the increase in paracellular permeability induced by enteropathogenic *Escherichia coli*. We used the human colon T84 cell line infected with a wild-type enteropathogenic *E. coli* (strain E2348/69). Paracellular permeability was followed by monitoring transepithelial electrical resistance variations and by observing zonula occludens-1 distribution. Two infection procedures were used: co-incubation (the pathogenic and probiotic strains were simultaneously incubated with T84 cells) and post-infection (the probiotic was added in the presence of pathogenic bacteria 3 h after the beginning of the infection). We also investigated the effect of *L. casei* on enteropathogenic *E. coli* adhesion. *L. casei* DN-114 001 inhibited, in a dose-dependent-manner, the decrease in enteropathogenic *E. coli*-induced transepithelial electrical resistance and zonula occludens-1 redistribution using two different infection procedures. However, *L. casei* did not inhibit pathogenic strain adhesion. *L. casei* DN-114 001 inhibited the increase in EPEC-induced paracellular permeability. This property could partially explain the previously observed health benefits of this probiotic for human natural defenses, such as those associated with prevention of diarrhea.

Keywords: *Lactobacillus casei* DN-114 001; Enteropathogenic *Escherichia coli*; Paracellular permeability; Adhesion properties; Probiotics; Natural defenses

1. Introduction

At the beginning of the last century, Russian immunologist Elie Metchnikoff [20] argued that the life-long intake of yogurt containing acid-producing microorganisms could explain the difference in longevity of some ethnic groups. The idea was that the bacteria in fermented products competed with microorganisms injurious to health. At present, it is known that the normal human microflora is an important barrier against colonization by exogenous pathogenic microorganisms, and that some bacteria of food origin, namely probiotics, also contribute to this barrier function. Probiotics, defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host", are indeed a science of the future as well as a reflection of the past [6].

Probiotics have been shown to exert several beneficial effects such as maintaining normal balanced intestinal microflora, modulating the immune system, detoxifying colonic contents, lowering serum cholesterol levels, promoting lactose tolerance, and producing metabolites essential to maintaining intestinal health [30].

The most commonly used microorganisms are lactic-acid-producing bacteria (LAB) such as the lactobacillus spe-

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cies. This family includes *Lactobacillus casei* DN-114 001 (*L. casei* DN-114 001), which has been shown, using a model of mice harboring human flora [23], to survive and initiate protein synthesis during its transit through the digestive tract. Several animal and human studies have demonstrated that this probiotic strain is beneficial in different intestinal pathological situations [9,13,32], and in modulating several intestinal functions [2,3,5,12]. In addition, milk fermented with *L. casei* DN-114 001 was shown to be effective in reducing the incidence and duration of diarrhea episodes in children, strengthening the natural host defense mechanisms, directly or indirectly associated with intestinal microflora [24,25].

Enteropathogenic *Escherichia coli* (EPEC) causes prolonged watery diarrhea in children in developing countries, and is occasionally recognized as an agent of diarrhea in outbreaks among children and adults in developed countries. EPEC colonizes the small bowel and adheres intimately to the apical enterocyte surface [15,28]. During this infection step, EPEC exploits the host cell signaling transduction pathway to produce an attaching and effacing lesion in which enterocyte microvilli are destroyed. It also induces several changes in the host cell [11], such as an increase in intracellular calcium concentration [1] and phosphorylation of several proteins (the most prominent being the myosin light chain, or MLC) [17]. Philpott et al. [26] showed that intestinal paracellular permeability increased in EPEC-infected T84 cells 3 h after the beginning of infection. For this purpose, they followed the decrease in transepithelial electrical resistance (TER) and zonula occludens-1 (ZO-1) distribution. Later, using the same in vitro model, it was demonstrated that EPEC-induced MLC phosphorylation was involved in the disturbance of the intestinal barrier function [34], which is partly controlled by tight junctions (TJs).

TJs seal the space between adjacent cells, limiting the diffusion of solutes through the intercellular space and creating a boundary between the apical and basolateral sides of cellular barriers such as epithelia [31]. TJs consist of integral membrane proteins (e.g., occludin, claudins, and junctional adhesion molecules) and cytoplasmic PDZ-domain-containing proteins (e.g., ZO-1, -2, -3, MAGUK, and the PAR family). ZO-1 and ZO-2 link tight junctions to the actin cytoskeleton by binding transmembrane proteins and actin to their N- and C-termini, respectively. This complex links TJs to a perijunctional actinomyosin ring, which supports and regulates TJ permeability [10].

Based on these data and on the previously described effects of *L. casei* DN-114 001 on intestinal natural defenses, we used the EPEC-infected human T84 cell line to evaluate the *L. casei* DN-114 001 capacity to modulate intestinal permeability in vitro. The T84 cell line was the first model used to show that TER is modified in the presence of an EPEC strain [26].

Although previous studies have been performed using probiotics and gut barrier function models, few are known to regulate intestinal paracellular permeability [16], one of the mechanisms by which *L. casei* DN-114 001 may beneficially influence the intestinal barrier function and consequently host natural defenses.

We studied the effect of different concentrations of *L. casei* DN-114 001 on the EPEC-induced decrease in TER and ZO-1 distribution using co-incubation and post-infection experimental procedures. In addition, we investigated whether *L. casei* has an effect on EPEC adhesion, the first step in infection.

2. Materials and methods

2.1. Cell line, media and bacterial strains

The human colon T84 cell line was obtained from the European Collection of Animal Cell Cultures (Salisbury, England). The T84 culture medium contained a 1:1 mixture of Dulbecco–Vogt modified Eagle medium and Ham's F-12 medium (DMEM-F12) supplemented with 50 µg of penicillin and 50 µg of streptomycin (Sigma) per ml and 5% fetal bovine serum (DAP). The enteropathogenic *E. coli* wild-type (WT) strain E2348/69 (kindly provided by J. Kaper, Center for Vaccine Development, University of Maryland, Baltimore) was grown overnight in Luria–Bertani medium at 37 °C without shaking. The probiotic strain, *L. casei* DN-114 001, provided by Danone Vitapole (France), was grown overnight at 37 °C in Mann Rogosa Sharpe (MRS) broth (Difco), without shaking.

2.2. Co-incubation and post-infection experimental procedures

We used two different experimental procedures, co-incubation (*L. casei* DN-114 001 and EPEC were simultaneously added to T84 apical surface) and post-infection (*L. casei* was added 3 h after the beginning of EPEC infection). Prior to bacterial incubations, the T84 medium was changed to a medium without serum and antibiotics (DMEM-F12). For infection purposes, approximately 10^8 EPEC (MOI = 100 bacteria/cell) were added to the apical surface of T84 monolayers and incubated at 37 °C in a 5% CO_2 water-jacketed incubator [34]. When infection was performed in the presence of *L. casei* DN-114 001, different quantities of probiotic were tested: 10^6, 10^7, and 10^8 CFU/ml (MOI = 1, 10, and 100 bacteria/cell, respectively). At the indicated times, TER was measured and at the end of infection, bacterial adhesion and ZO-1 distribution were observed as described hereafter.

2.3. Inhibitor

MLC kinase (MLCK) inhibitor ML-7 (Calbiochem, Meudon, France) was stored in 50% ethanol at 4 °C. In co-incubation experiments, cells were pre-incubated for 60 min with ML7 (1 nM), before infection. In post-infection tests,