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Research paper

Effect of probiotic bacteria on phagocytosis and respiratory burst activity of blood polymorphonuclear leukocytes (PMNL) in mice infected with *Trichinella spiralis*

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ABSTRACT

This study focusses on the effect of probiotic (bacteriocinogenic) strains on parasite infection and innate immunity – phagocytosis and oxidative burst of blood monocytes and polymorphonuclear leukocytes (PMNL) in mice infected with *Trichinella spiralis*. Bacteriocinogenic and probiotic strains of different origin (*Enterococcus faecium* AL41 = CCM8558, *Enterococcus durans* ED26E/7, *Lactobacillus fermentum* AD1 = CCM7421, *Lactobacillus plantarum* 17L/1) were administered daily in dose of 10^9 CFU/ml in 100 μl and mice were infected with 400 larvae of *T. spiralis* on 7th day of treatment. Phagocytic activity of blood leukocytes was inhibited at week 3 and 4 post infection (p.i.), i.e. in the time of massive muscle invasion with larvae *T. spiralis*. Administration of bacterial strains to mice prior to *T. spiralis* infection elevated and prolonged phagocytic activity of blood leukocytes and their ingestion capability from week 1 to 3 of the infection and the phagocytosis was inhibited only at week 4 p.i. The highest stimulative effect on phagocytosis was induced by strains *E. durans* ED26E/7, *L. fermentum* AD1 = CCM7421, and *L. plantarum* 17L/1. The percentage of cells with respiratory burst and their enzymatic activity was increased after *T. spiralis* infection with the exception of week 3 p.i. In contrast, in all mice treated with bacterial strains the enzymatic stimulation was observed after the infection, with the highest intensity caused by strains *E. durans* ED26E/7, *L. fermentum* AD1 = CCM7421 and *L. plantarum* 17L/1. The administration of probiotic strains stimulated phagocytosis and respiratory burst of blood PMNL that could contribute to a decreased larval migration and a destruction of muscle larvae and then reduced parasite burden in the host. The protective effect against *T. spiralis* infection was induced by all strains, but the highest reduction was recorded by *E. faecium* AL41 = CCM8558.

1. Introduction

The intestinal parasites interact with gut microbiota and the host immune system. On the other hand, gut microbiota represents a relevant factor that may strongly interfere with the pathophysiology of parasitic infections, determine the parasite survival and the outcome of parasitic infections. Therefore probiotics can play an important role in reducing the pathogenicity of many parasites (Berrilli et al., 2012). During the last decade, probiotics as a means for the control of parasite infections were recorded mainly in intestinal diseases, but also in non-gut infections (Travers et al., 2011). Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002). The mechanism of action of probiotics is related to their ability to compete with pathogens for adhesion sites, to enhance mucosal barrier activity, to produce antibacterial agents known as bacteriocins or to modulate the host's immune response, although these abilities are strain-dependent (Donelli et al., 2013; Butel, 2014).

An important mechanism, whereby probiotic bacteria may provide a health benefit, is by modulating immune responses. Generation of an effective immune response is a complex, coordinated process that requires many different kinds of cells, including neutrophils, macrophages, natural killers, dendritic cells, and lym-

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phocytes, which act in a specific and coordinated manner to mount a potentially lethal immune response against invading pathogens (Mogensen, 2009). Phagocytosis by polymorphonuclear leukocytes constitutes an essential arm of host defence against pathogens and also parasite infections (Hübel et al., 2002). The phagocytic process can be separated into several major stages: chemotaxis (migration of phagocytes to inflammatory sites), attachment of particles to the cell surface of phagocytes, ingestion (phagocytosis) and intracellular killing by oxygen-dependent and oxygen-independent mechanisms (Aderem, 2003).

This study describes an investigation of the relationship between probiotic bacteria, the parasite *Trichinella spiralis* and its host from the standpoint of innate immunity. The nematode *T. spiralis* has been chosen as a model parasite to verify antiparasitic and immunomodulatory properties of probiotic and bacteriocin-producing strains. *T. spiralis* causes an intestinal and tissue disease (Bruschi and Murrell, 2002) – trichinellosis is characterized by enteritis (induced by adult worms) and inflammation with degenerative changes in the skeletal muscles (induced by larvae). Parasite-induced formations of larval capsule and host immunosuppression are two major hallmarks of *Trichinella* spp. infections, but the molecular mechanisms mediating these processes remain unknown. Host immunosuppression could be regulated in the intestinal phase by the serine protease from adults and newborn larvae (strong suppression) and in the muscle phase by the serine protease from muscle larvae (moderate suppression) (Wu et al., 2013). *Trichinella* can modulate dendritic cells function and induce immunosuppression including regulatory T and B cells, alternatively activated macrophages, and cytokine production. In this way, helminths suppress and control immunopathology in the host (Aranzamendi et al., 2013). The pathology of trichinellosis is particularly a reaction to the initial inflammatory response during the intestinal phase and to the subsequent allergic and inflammatory responses during larval migration and invasion of the host muscles (Bruschi and Chiumiento, 2011). The chemotherapy of human trichinellosis with available anthelmintics (benzimidazoles) is active only against adult worms, but not against muscle larvae. Efforts to develope vaccines against trichinellosis are not successful due to the diversity of stage-specific antigens, immune-evasion strategies and the modulatory effect of host responses (Patel et al., 2009; Ortega-Pierres et al., 2015). As trichinellosis is an important parasitic zoonosis, with a worldwide distribution and epidemic occurrence (Devleesschauwer et al., 2015), the need for developing new methods for controlling this disease is necessary and the use of the immunostimulant probiotic bacteria has been proposed (Martínez-Gómez et al., 2011; Temsahy et al., 2015).

The present study was designed to study the effects of four different probiotic strains of lactobacilli and enterococci on blood leukocytes and monocytes functions in mice after *T. spiralis* infection. Cell functions were assessed in terms of phagocytosis and respiratory burst activity; antiparasitic properties of probiotic strains by the parasite burden.

2. Materials and methods

2.1. Probiotic strains

The effect of the following bacteria was tested: bacteriocin-producing strains with probiotic properties (*Enterococcus faecium* AL41 = CCM8558, *Enterococcus durans* ED26E/7, and *Lactobacillus plantarum* 17L/1) and probiotic strain *Lactobacillus fermentum* AD1 = CCM7421.

Enterococcus faecium AL41 = CCM8558 (isolated and characterized at the Institute of Animal Physiology SAS-IAP SAS, Košice, Slovakia and deposited in the Czech Culture Collection of Microorganisms, Brno, Czech Republic – CCM8558) is an environment-derived strain. It was prepared for experimental work at IAP SAS, Košice, Slovakia. The strain produces a new enterocin (Ent M) with a wide antimicrobial inhibitory spectrum and possesses probiotic properties, i.e. stimulates unspecific immunity in the host (Lauková et al., 1998, 2012, 2015a; Mareková et al., 2007).

Enterococcus durans ED26E/7 was isolated from traditional ewes milk lump cheese at the Research Dairy Institute, Žilina; RDI, Žilina, Slovakia) but identified, characterized and prepared for experiment at IAP SAS, Košice, Slovakia (Lauková et al., 2015b).

Lactobacillus fermentum AD1 = CCM7421 was isolated and characterized at IAP SAS, Košice, Slovakia and deposited in the Czech Culture Collection of Microorganisms, Brno, Czech Republic. It is a canine-derived strain possessing probiotic properties (Strompfová et al., 2008, 2012).

Lactobacillus plantarum 17L/1 was isolated from stored ewes cheese (RDI, Žilina, Slovakia) but identified, characterized and prepared for experiment at IAP SAS, Košice, Slovakia (Lauková et al., 2013).

All strains were first tested according to the EFSA rules (Piskoríková, 2010). For the experiment they were prepared as follows: they were cultivated in MRS broth (Merck, Eppelheim, Germany) at 37 °C for 24 h. Broth cultures were centrifuged (30 min at 10,000*g*) and the supernatant was resuspended in Ringer solution (Merck, pH 7.0) to a concentration 10^9 colony forming unit per ml (CFU/ml). The purity of the strains was checked by the spreading of dilutions onto the selective media ME-*Enterococcus* agar (Difco, Thermo Fisher Scientific, Roskilde, Denmark) and/or MRS agar (Merck). The cultures concentration was stable for 1 week at 4 °C.

2.2. Parasite

The reference isolate of *Trichinella spiralis* (ISS 004) (obtained and assigned codes from the Trichinella Reference Centre in Rome), maintained by serial passage in ICR mice at the Institute of Parasitology SAS, was used for the infection. Larvae were released by artificial digestion (1% pepsin, 1% HCl for 4 h at 37 °C) of tissue following the protocol of Kapel and Gamble (2000) and kept in saline solution until inoculation of experimental mice.

2.3. Experimental design

The experiment was carried out on pathogen-free 8 weeks old male BALB/c mice (VELAZ, Prague, Czech Republic; n = 196) weighting 18–20 g. Mice were kept under a 12-h light/dark regime at room temperature (22–24 °C) and 56% humidity on a commercial diet and water. The experimental protocol complied with current Slovak ethics law and it was approved by the Animal Care Committee of the Institute of Parasitology SAS and the State Veterinary and Food Administration of the Slovak Republic (No. 4296/12-221d).

Animals were divided randomly into six groups as follows: Control (n = 21) – without the administration of bacterial strains and uninfected; Group 1 (n = 35) – *T. spiralis* infection; Group 2 (n = 35) – *E. faecium* AL41 = CCM8558 + *T. spiralis*; Group 3 (n = 35) – *E. durans* ED26E/7 + *T. spiralis*; Group 4 (n = 35) – *L. fermentum* AD1 = CCM7421 + *T. spiralis;* Group 5 (n = 35) – *L. plantarum* 17L/1+ *T. spiralis*. Probiotic strains were administered *per os* daily at a dose of 10^9 CFU/ml in 100 μl and mice were infected *per os* with 400 *T. spiralis*/mouse on 7th day of treatment. Samples of blood, the small intestines and muscles were obtained from three mice of control and five of infected groups on days: −7, 0 (prior infection), 5, 11, 18, 25, and 32 post infection (p.i.).

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