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МОДУЛЬНОЕ ВЛИЯНИЕ ПРОБИОТИЧЕСКОЙ ТЕРАПИИ НА КИШЕЧНЫЕ ЛИМФОЦИТЫ У МЫШЕЙ, ЗАРАЖЕННЫХ *TRICHINELLA SPIRALIS*

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Аннотация

Важными компонентами иммунитета слизистой оболочки кишечника являются свободные интраэпителиальные лимфоциты и *lamina propria* лимфоциты, участвующие в регуляции и активности иммунного ответа. Это исследование выявило присутствие вспомогательных CD4 и цитотоксических CD8 Т-лимфоцитов и В-лимфоцитов в тонкой кишке мышей, получавших пробиотические штаммы и инфицированных *Trichinella spiralis.* Штаммы бактерий различного происхождения (*Enterococcus faecium* CCM8558, *Enterococcus durans* ED26E/7, *Lactobacillus fermentum* CCM7421, *Lactobacillus plantarum* 17L/1) вводили ежедневно в дозе 10^9 К ФЕ/мл в 100 мкл, и мыши были заражены 400 личинками *T. spiralis* на 7-й день лечения. *L. fermentum* CCM7421 и *L. plantarum* 17L/1 увеличивали количество хелперных CD4 Т-клеток в эпителии и цитотоксических CD8 Т-клеток в собственной пластинке слизистой оболочки на 7-й день введения (до паразитарной инфекции). Инфекция *T. spiralis* вызывала значительное ингибирование исследуемых субпопуляций лимфоцитов с 5-го по 25-й день после заражения (п.з.). Лактобациллы восстанавливали количество CD4 Т-клеток в эпителии и собственной пластинке на уровне здорового контроля с 11 дня п.з. Все штаммы стимулировали количество CD8 Т-клеток у инфицированных мышей, но по сравнению с контролем, CD8 Т-клетки были уменьшены в эпителии до 25 дня п.з. и в

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собственной пластинке только на 5 день п.з. Пробиотическая терапия не влияла на ингибирование В-клеток (CD19) в тонкой кишке после инфекции *T. spiralis* до 25 дня п.з., но стимуляция В-клеток была обнаружена после введения *E. durans* ED26E/7 и *L. fermentum* CCM7421 на 32 день п.з. Полученные результаты подтвердили штаммоспецифический иммуномодулирующий эффект пробиотических бактерий. Наибольший иммуномодулирующий потенциал для CD4 и CD8 Т-лимфоцитов кишечника во время инфекции *T. spiralis* был п подтвердили *L. fermentum* CCM7421 и *L. plantarum* 17L/1. Штаммы *E. faecium* CCM8558 и *E. durans* ED26E/7 активировали только цитотоксические CD8 Т-клетки в собственной пластинке слизистой оболочки.

Ключевые слова: пробиотические бактерии, лимфоциты, лимфоциты собственной пластинки, *Trichinella spiralis*.

MODULATORY EFFECT OF PROBIOTIC THERAPY ON INTESTINAL LYMPHOCYTES IN MICE INFECTED WITH *TRICHINELLA SPIRALIS*

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Abstract

Important components of the intestinal mucosal immunity are free intraepithelial and *lamina propria* lymphocytes involved in the regulation and activity of the immune response. This study detected the presence of helper CD4 and cytotoxic CD8 T lymphocytes, and B lymphocytes in the small intestine of mice treated with probiotic strains and infected with *Trichinella spiralis*. Bacterial strains of different origin (*Enterococcus faecium* CCM8558, *Enterococcus durans* ED26E/7, *Lactobacillus fermentum* CCM7421, *Lactobacillus plantarum* 17L/1) were administered daily in

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dose of 10^9CFU/ml in 100 μl and mice were infected with 400 larvae of *T. spiralis* on 7th day of treatment. *L. fermentum* CCM7421 and *L. plantarum* 17L/1 increased numbers of helper CD4 T cells in the epithelium and cytotoxic CD8 T cells in the lamina propria on 7th day of administration (before parasitic infection). *T. spiralis* infection caused a significant inhibition of examined lymphocyte subpopulations from 5 to 25 days post infection (p.i.). Lactobacilli restored the CD4 T cell numbers in the epithelium and lamina propria on the level of healthy control from day 11 p.i. All strains stimulated the numbers of CD8 T cells in infected mice, but in comparison to control, CD8 T cells were reduced in the epithelium until day 25 p.i. and in the *lamina propria* only on day 5 p.i. An inhibition of B cells (CD19) in the small intestine after *T. spiralis* infection was not affected by probiotic therapy till day 25 p.i., but a stimulation of B cells was found after treatment with *E. durans* ED26E/7 and *L. fermentum* CCM7421on day 32 p.i. The obtained results confirmed the strain-specific immunomodulatory effect of probiotic bacteria. The greatest immunomodulatory potential on the gut CD4 and CD8 T lymphocytes during *T. spiralis* infection was confirmed by *L. fermentum* CCM7421 and *L. plantarum* 17L/1. Strains *E. faecium* CCM8558 and *E. durans* ED26E/7 activated only cytotoxic CD8 T cells in the *lamina propria*.

Keywords: probiotic bacteria, intraepitelial lymphocytes, *lamina propria* lymfocyty, *Trichinella spiralis*.

Introduction. The nematode *Trichinella spiralis* causes an intestinal and tissue disease – trichinellosis characterized by the enteritis (induced by adult worms) and the inflammation with degenerative changes in the skeletal muscles (induced by larvae). Effector mechanisms against *Trichinella* are dependent on T cells that induce inflammatory changes, cytokine and antibody response. The gut epithelium includes a heterogeneous T lymphocyte population, most of which are cytotoxic CD8 T cells, furthermore helper CD4 T cells, and regulatory T cells. In the *lamina propria*, there is present a large number of B cells and IgA-producing plasma cells, CD4 T cells, macrophages and dendritic cells.

The intestinal microbiota significantly affects the development, maturation, and modulation of the immune system in infections. Probiotic strains with a health benefit are able to inhibit and compete with pathogens, enhance mucosal barrier activity, modulate the host immune response and have an anti-parasitic effect. Gut microbiota strongly interfere with the pathophysiology of parasitic infections, determine the parasite survival and the outcome of parasitic infections. On these bases, there is a growing interest in explaining the interactions between the microbiota, immune response, inflammatory processes, and intestinal parasites. The aim of this study was to detect the presence of helper CD4 and cytotoxic CD8 T lymphocytes,

and B lymphocytes in the small intestine of mice after probiotic therapy and *Trichinella spiralis* infection.

Materials and methods. Animals (BALB/c mice) were divided into 6 groups: Control (n = 21) – healthy mice without treatment and infection; Group 1(n = 21) – *T. spiralis* infection without the administration of bacterial strains; Group 2 (n = 21) – *E. faecium* CCM8558 + *T. spiralis*; Group 3 (n = 21) – *E. durans* ED26E/7 + *T. spiralis*; Group 4 (n = 21) – *Lactobacillus fermentum* CCM7421 + *T. spiralis*; Group 5 (n = 21) – *L. plantarum* 17L/1 + *T. spiralis*. Probiotic strains were administered *per os* daily at a dose of 109 CFU/ml in 100 µl and mice were infected *per os* with 400 *T. spiralis* larvae/mouse on day 7 of treatment. Probiotic strains (Institute of Animal Physiology, Košice): *E. faecium* CCM8558 is an environment-derived strain producing enterocin M. *E. durans* ED26E/7 was isolated from traditional ewes milk lump cheese, producing durancin-like bacteriocin. *L. plantarum* 17L/1 was isolated from stored ewes cheese. *L. fermentum* AD1 = CCM7421 is a canine-derived strain.

Intra-Epithelial (IEL) and Lamina Propria Lymphocytes (LPL) were isolated and purified by the modified method of Solano-Aguilar et al. (2000). Immunophenotyping of lymphocytes was determined by direct immunofluorescence. Lymphocytes were labelled with rat anti-murine CD4+ FITC (fluorescein isothiocyanate), CD8+ PE (phycoerythrin) and CD19+ FITC conjugated monoclonal antibodies. The cells were measured and analysed by a flow cytometer FACScan, with software Cell Quest. The results were processed with one-way ANOVA and *post hoc* Tukey test.

Results. A significant increase in the number of helper CD4 T cells in the epithelium and cytotoxic CD8 T cells in the *lamina propria* was already detected after 7 days of administration of lactobacilli (before the parasitic infection). During the intestinal and early muscle phase of *T. spiralis* infection there was found a significant decrease in lymphocyte subpopulations in the epithelium and *lamina propria* of the small intestine. Lactobacilli restored the CD4 T cell numbers in the epithelium and lamina propria on the level of healthy control from day 11 p.i. Enterococci had no influence on CD4 T lymphocytes, except of day 11 p.i., when these cells were stimulated in the *lamina propria* to the control level. The CD4 T-cell subset plays a key role in worm expulsion from the gut, because they regulate the gut physiology (e.g. inflammation, hypercontractility, mucus hypersecretion), but CD8 T cells are not associated with this process.

A significant stimulation of intraepithelial CD8 lymphocytes was recorded after the administration of all probiotic strains on days 11 and 18 p.i.,

however, they not reached the level of control. After administration of lactobacilli and enterococci, the number of CD8 T cells was normalized to the level of healthy mice in the gut epithelium on days 25 and 32 p.i., whereas in the *lamina propria* these cells were restored from day 11 p.i. Transfer of cytotoxic CD8 T cells from the *lamina propria* to the intestinal epithelium can contribute to antiparasitic defense and reduce the number of larvae in the host.

The increased numbers of both T cell subpopulations during the muscle phase of trichinellosis could be a result of the development of host immune response to adult and larval antigens and development of memory CD4, CD8 T lymphocytes, and B lymphocytes.

The presence of CD19 B cells in the small intestine was significantly reduced in all experimental groups till day 11 p.i., afterward their numbers in the epithelium and *lamina propria* increased slightly. Newborn larvae can be destroying with the mechanism of antibody-dependent cell-mediated cytotoxicity before they reach the muscles. A stimulation of B cells after treatment with *E. durans* ED26E/7 and *L. fermentum* CCM7421was found on day 32 p.i.

Conclusion. Changes in IEL and LPL subpopulation after probiotic therapy indicate a positive modulation of the gut immunity in *T. spiralis* infection and perspective use of tested probiotic strains in therapy of trichinellosis. An activation of the T cells in the small intestine of mice infected with *T. spiralis* can contribute to worm expulsion from the gut and stimulated an anti-parasitic immune response also in the muscle phase of infection.

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References

1. Solano-Aguilar G.I., Vengroski K.G., Beshah E. et al. J. Immunol. Methods 2000; 241: 185–199.