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# РЕГУЛЯТОРНЫЕ Т-КЛЕТКИ ЛИМФАТИЧЕСКИХ УЗЛОВ У Muc2$^{-/-}$ МЫШЕЙ С *HELICOBACTER* SPP.

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**Резюме.** Иммунные процессы, связанные с формированием устойчивости к патогенам в кишечнике, зависят от микробиома. Поддержание гомеостаза в кишечнике обеспечивают регуляторные Т-клетки. При воспалительных заболеваниях кишечника (ВЗК) наблюдают как нарушение функции Т-регуляторных клеток, так и изменения микрофлоры. Развитие осложнений при этих заболеваниях сопровождается различными инфекциями. Однако некоторые представители патобионтов, например *Helicobacter* spp., могут влиять на Т-регуляторные клетки. Одной из генетических моделей для изучения ВЗК являются мыши с нокаутом гена Muc2. У таких мышей, как и у людей с ВЗК, эпителиальные и иммунные клетки кишечника находятся в тесном взаимодействии с микрофлорой. Полагают, что иммунные клетки лимфатических узлов Muc2$^{-/-}$ мышей чувствительны к изменению сформировавшейся у них микрофлоры, даже если в ее состав входят патобионты. В данном исследовании было показано влияние присутствия *Helicobacter* spp. на количество и процент различных типов лейкоцитов и Т-регуляторных клеток в мезентериальных лимфатических узлах Muc2$^{-/-}$ мышей. Количество CD45$^+$CD19$^+$, CD45$^+$CD3$^+$, CD45$^+$CD3$^+$CD4$^+$, CD45$^+$CD3$^+$CD8$^+$-клеток в мезентериальных лимфатических узлах мышей Muc2$^{-/-}$ был достоверно выше, чем у мышей дикого типа Muc2$^{+/+}$. Однако наличие инфекции у Muc2$^{-/-}$ мышей отменяло увеличение количества CD45$^+$CD19$^+$, CD45$^+$CD3$^+$, CD45$^+$CD3$^+$CD4$^+$, CD45$^+$CD3$^+$CD8$^+$-клеток. У мышей дикого типа Muc2$^{+/+}$ инфекция не оказывала достоверного эффекта на клетки в мезентериальных лимфатических узлах. Такое изменение в снижении иммунных клеток в мезентериальных лимфатических узлах под действием *Helicobacter* spp. может быть связано с активацией регуляторных Т-клеток. Действительно, было показано, что наличие врожденной инфекции *Helicobacter* spp. вызывало увеличение количества регуляторных Т-клеток (CD45$^+$CD4$^+$CD25$^+$FoxP3$^+$) в мезентериальных лимфатических узлах. Известно, что регуляторные Т-клетки обеспечивают противовоспалительные реакции в кишечнике. Таким образом, увеличение регуляторных Т-клеток способствует снижению всех типов иммунных клеток в мезентериальных лимфатических узлах мышей Muc2$^{-/-}$ с инфекцией *Helicobacter* spp., что способствует улучшению жизнедеятельности этих мышей и, возможно, уменьшает воспалительные реакции в кишечнике. Это может быть свидетельством того, что некоторые патобионты, приобретенные с рождения, могут быть активаторами регуляторных механизмов иммунитета и, тем самым, оказывать благоприятное воздействие на хозяина.

*Ключевые слова: Т-регуляторные лимфоциты, муцин 2, микрофлора, Helicobacter, мыши*

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# LYMPH NODE REGULATORY T-CELL IN Muc2$^{-/-}$ MICE WITH *HELICOBACTER* SPP.

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**Abstract.** The immune processes associated with the formation of resistance to pathogens in the intestine depend on the microbiome. The maintenance of homeostasis in the intestine is provided by regulatory T-cells. In inflammatory bowel disease (IBD), both a disturbance of the T-regulatory function and changes in microflora are observed. Aggravation of the disease is accompanied by various infections. However, pathobionts such as *Helicobacter* spp., can affect regulatory T-cells. One of the genetic models for studying IBD is Muc2 knockout mice. In these mice, as in humans with IBD, intestinal epithelial and immune cells closely interact with the microflora. It is believed that the immune cells of the lymph nodes Muc2$^{-/-}$ mice are sensitive to changes in the microflora formed in them. In this study, the effect of *Helicobacter* spp. on the number and percentage of different types of leukocytes and T regulatory cells in the mesenteric lymph nodes of Muc2$^{-/-}$ mice was studied. The number of CD45$^+$CD19$^+$, CD45$^+$CD3$^+$, CD45$^+$CD3$^+$CD4$^+$, CD45$^+$CD3$^+$CD8$^+$-cells in the mesenteric lymph nodes of Muc2$^{-/-}$ mice was significantly higher to compare with wild-type Muc2$^{+/+}$ mice. However, the presence of infection in Muc2$^{-/-}$ mice canceled the increase in the number of CD45$^+$CD19$^+$, CD45$^+$CD3$^+$, CD45$^+$CD3$^+$CD4$^+$, CD45$^+$CD3$^+$CD8$^+$-cells. In wild-type Muc2$^{+/+}$ mice, infection had no significant effect on cells in mesenteric lymph nodes. This change in the decrease in immune cells in the mesenteric lymph nodes under the *Helicobacter* spp. may be associated with the activation of regulatory T-cells. Indeed, it has been shown that the presence of a congenital *Helicobacter* spp. infection increased of the number of regulatory T-cells (CD45$^+$CD4$^+$CD25$^+$FoxP3$^+$) in the mesenteric lymph nodes. Well known that regulatory T-cells mediate anti-inflammatory responses in the gut. Thus, an increase in regulatory T-cells promotes a decrease in all types of immune cells in the mesenteric lymph nodes of Muc2$^{-/-}$ mice infected with *Helicobacter* spp. It could provide an improvement in the vital functions of these mice and possibly reduces inflammatory responses in the intestine. This may indicate that some congenital pathobionts activate of the regulatory mechanisms of immunity and, thereby, have a beneficial effect on the host.

*Keywords: regulatory T-cells, mucine 2, microflora, Helicobacter, mice*

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## Introduction

Microflora plays an important role in the formation of intestinal lymphoid tissue, to increase resistance to pathogens [8]. Regulatory T-cells controlled by symbiotic microflora are involved in intestinal homeostasis [4, 9]. It is known that in germ-free vs. conventional mice (GF) regulatory T-cells have less suppressive activity [13]. Certain bacterial species can influence on the function of regulatory T-cells. *Bacteroides fragilis* stimulates the production of anti-inflammatory cytokine IL-10 by CD4$^+$-cells, preventing development of inflammation in mice [14]. The intestinal colonization of such bacteria in GF mice stimulates the differentiation of IL-10-producing CD4$^+$FoxP3$^+$T-cells [14]. *Clostridium* spp. leads to increased number of regulatory T-cells in the gut *lamina propria* and stimulates IL-10 production [3]. The differentiation of regulatory T-cells can also be influenced by bacterial metabolites. Butyrate has an effect on the expression of the FoxP3 transcription factor gene by CD4$^+$T-cells in mouse colon as well as *in vitro*. At the same time, dietary butyrate helps to ameliorates mouse induced colitis [7].

Muc2 knockout mice (Muc2$^{-/-}$) are one of the genetic models for studying intestinal inflammation [5]. At the same time, colitis in Muc2$^{-/-}$ mice has similar features to ulcerative colitis in humans [15]. Muc2$^{-/-}$ mice are more susceptible to various pathogens, due to impaired production of mucin2. In the presence of pathogens, these mice exhibit acute intestinal inflammation [5]. Thus, Muc2$^{-/-}$ mice as IBD model can be used to study a relationship between changes in intestinal microflora and intestinal inflammation.

Previously in our laboratory have been shown that mice with impaired gut barrier function and mutation in the gene Muc2 ($Muc2^{-/-}$ $Kaiso^{-/-}$), are more sensitive to treatment with antibiotics, and the elimination of Helicobacter spp, compared to mice with normal gut barrier function (C57BL/6). *Helicobacter* spp. are considered pathobionts, i.e. show their pathogenic properties only in the presence of defects in immune function, or under special environmental conditions. Also *Helicobacter* spp. can induce regulatory T-cells [10]. Member of the genus *Helicobacter* (*H. hepaticus*) enable mechanisms that maintain a non-pathogenic, symbiotic relationship with the host organism [6]. Thus, the bacteria *Helicobacter* spp. found in host from birth, can act as symbionts, influencing the formation of the immune system.

Due to the lacked mucin2, the intestinal epithelial and immune cells are in closer contact with the microflora. It can be assumed that the immune cells in regional lymph nodes of $Muc2^{-/-}$ mice are sensitive to changes in microbiota composition. In this study we examined an effect of inoculated *Helicobacter spp.* on number and percentage of different types of leukocytes and T-regulatory cells in the mesenteric lymph nodes (LN) of $Muc2^{-/-}$ mice.

## Materials and methods

The study was carried out on sexually mature female knockout Muc2 gene knockout mice ($Muc2^{-/-}$) and their littermates with normal gene function ($Muc2^{+/+}$). Mice were free of species-specific pathogens (SPF) recommended by the Federation of European Laboratory Animal Science Associations (FELASA), except for *Helicobacter spp*. Three to six females were housed in individually ventilated cages (Optimice, USA) under an artificial light regimen (14L: 10D), at a temperature of 20-22 °C. Animals received chop diet (Ssniff, Germany) and sterile drinking water *ad libitum*.

The study was carried out on four groups of mice (two genotypes with and without *Helicobacter* spp., n = 7 per each group). Mesenteric lymph node cells were isolated and stained with PE-anti-CD3ε, FITC-anti-CD4, PE / Cy7-anti-CD8a and PE-anti-CD3ε, FITC-anti-CD19, PE / Cy7-anti-CD4 and APC-anti-CD25, AlexaFluor488-anti-FoxP3 and True-NuclearTM Fix (BioLegend, USA) and analyzed on a Guava easyCyte 8HT Flow Cytometer (Merk, Germany).

Data statistical distribution was not normal; the analysis of the data was performed by using nonparametric criteria.

## Results and discussion

The effect of the groups on the cell number in mesenteric LNs (mLNs) was found (Kruskal–Wallis test $H(3,25) = 16.08$, $p < 0.01$). The cell number in mLN of mutant mice born without infection was higher than in $Muc2^{-/-}$ mice with infection, as well as in $Muc2^{+/+}$ mice of the corresponding infection status (Mann–Whitney U-test $Z = 3.03$ and $Z = 2.85$, $p < 0.01$; Figure 1A). A similar effect was observed for the number of lymphocyte subsets such as $CD19^+$, $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$ (Figure 1A).

The study of regulatory T-cell subpopulations revealed an effect of the group on the percentage of $CD25^+FoxP3^+$-cells among $CD4^+$-cells (Kruskal–

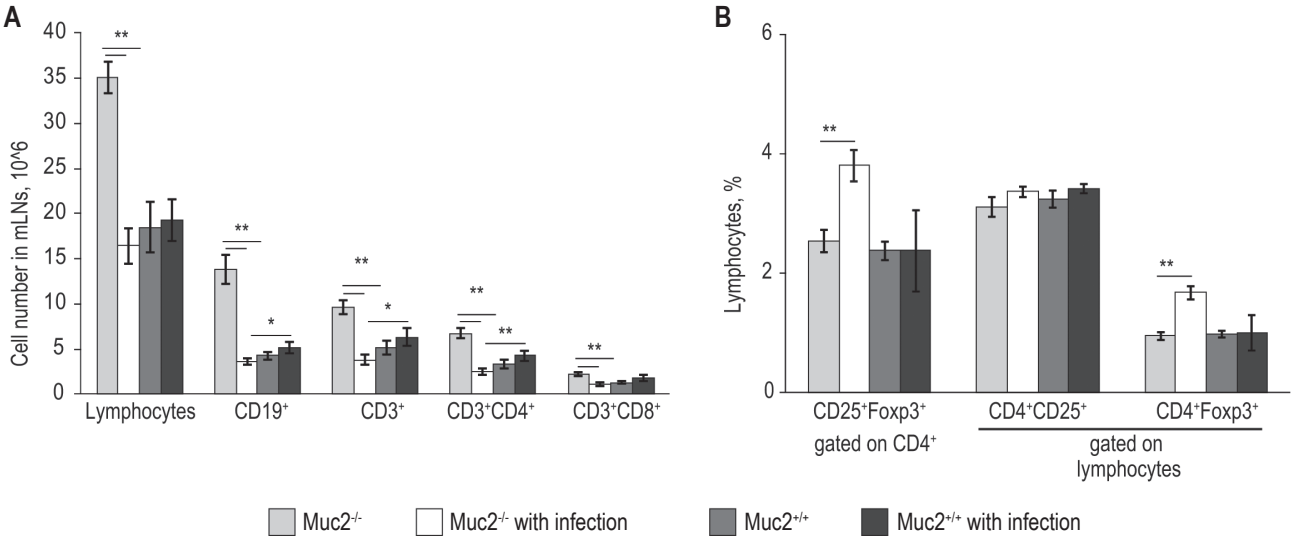


**Figure 1. Lymphocytes in mLNs of $Muc2^{-/-}$ and $Muc2^{+/+}$ mice**

Note. A, cell number of lymphocytes and different lymphocyte subsets in the mLNs. B, percentage of regulatory T-cell subpopulations. *, $p < 0.05$; **, $p < 0.01$; Mann–Whitney U-test.

Wallis test H(3,25) = 9.15, p < 0.05). The percentage of $CD25^+FoxP3^+$-cells among $CD4^+$-cells in $Muc2^{-/-}$ mice with vs. without infection was higher (Mann–Whitney U-test Z = 2.78, p < 0.01; Figure 1B). The increased number of such cells was probably due to upregulated expression of the FoxP3 protein in $CD4^+$-cells (effect of the Kruskal–Wallis test H(3.25) = 9.47, p < 0.05). The percentage of $CD4^+FoxP3^+$-cells among lymphocytes was higher in $Muc2^{-/-}$ mice with infection compared to mice without infection (Mann-Whitney U-test Z = 2.92, p < 0.01; Figure 1B). At the same time, no such effect was found for the percentage of $CD4^+CD25^+$-cells.

Thus, the presence of *Helicobacter* spp. in $Muc2^{-/-}$ mice was associated with increased mLN percentage of regulatory T-cells ensuring anti-inflammatory responses in the intestine.

A study of lymphocytes in mLNs showed that $Muc2^{-/-}$ mice bearing *Helicobacter* spp. had increased percentage of $CD25^+FoxP3^+$ regulatory T-cells compared to mice without infections. These differences are likely due to increased expression of the FoxP3 protein in $CD4^+$-cells. Possibly, *Helicobacter* spp. activates the regulatory function of T-cells in mice, which can prevent the development of extremely strong immune responses to host microflora. It was shown that tolerogenic properties of *Helicobacter* spp. particularly were due to its potential to stimulate the production of the anti-inflammatory cytokine IL-10 [2, 11] and activate regulatory T-cells [1, 10, 12]. It is possible that the pathobiont *Helicobacter* spp. can favorably influence the state of $Muc2^{-/-}$ mice by stimulating the regulatory function. In our study, we found that *Helicobacter* spp.-free $Muc2^{-/-}$mice had prolapse at an earlier age (2-3-months old) and significantly more frequently than in mice with infection. It was also noted that the fertility of $Muc2^{-/-}$ mice without *Helicobacter* spp. is lower than in mice with infection (unpublished data).

Thus, the presence of *Helicobacter* spp. in $Muc2^{-/-}$ mice, it was associated with an increase in regulatory T-cells, which provide anti-inflammatory responses in the intestine and improve the vital functions of mice with a pathology of thinned mucus in the intestine.

## References

1. Altobelli A., Bauer M., Velez K., Cover T.L., Müller A. Helicobacter pylori vacA targets myeloid cells in the gastric lamina propria to promote peripherally induced regulatory T-Cell differentiation and persistent infection. *MBio, 2019, Vol. 10, no. 2, e00261-19.* doi: 10.1128/mBio.00261-19.

2. Arnold I.C., Hutchings C., Kondova I., Hey A., Powrie F., Beverley P., Tchilian E. Helicobacter hepaticus infection in BALB/c mice abolishes subunit-vaccine-induced protection against M. tuberculosis. *Vaccine, 2015, Vol. 33, no. 15, pp. 1808-1814.*

3. Atarashi K., Tanoue T., Shima T., Imaoka A., Kuwahara T., Momose Y., Cheng G., Yamasaki S., Saito T., Ohba Y., Taniguchi T., Takeda K., Hori S., Ivanov I.I., Umesaki Y., Itoh K., Honda K. Induction of colonic regulatory T cells by indigenous clostridium species. *Science, 2011, Vol. 331, no. 6015, pp. 337-341.*

4. Barnes M.J., Powrie F. Regulatory T-cells reinforce intestinal homeostasis. *Immunity, 2009, Vol. 31, no. 3, pp. 401-411.*

5. Bergstrom K.S.B., Kissoon-Singh V., Gibson D.L., Ma C., Montero M., Sham H.P., Ryz N., Huang T., Velcich A., Finlay B.B., Chadee K., Vallance B.A. Muc2 protects against lethal infectious colitis by disassociating pathogenic and commensal bacteria from the colonic mucosa. *PLoS Pathog., 2010, Vol. 6, no. 5, e1000902.* doi: 10.1371/journal.ppat.1000902.

6. Chow J., Mazmanian S.K. A Pathobiont of the microbiota balances host colonization and intestinal inflammation. *Cell Host Microbe, 2010, Vol. 7, no. 4, pp. 265-276.*

7. Furusawa Y., Obata Y., Fukuda S., Endo T. A., Nakato G., Takahashi D., Nakanishi Y., Uetake C., Kato K., Kato T., Takahashi M., Fukuda N.N., Murakami S., Miyauchi E., Hino S., Atarashi K., Onawa S., Fujimura Y., Lockett T., Clarke J.M., Topping D.L., Tomita M., Hori S., Ohara O., Morita T., Koseki H., Kikuchi J., Honda K., Hase K., Ohno H. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T-cells. *Nature, 2013, Vol. 504, no. 7480, pp. 446-450.*

8. Gensollen T., Iyer S.S., Kasper D.L., Blumberg R.S. How colonization by microbiota in early life shapes the immune system. *Science, 2016, Vol. 352, no. 6285, pp. 539-544.*

9. Geuking M.B., Cahenzli J., Lawson M.A.E., Ng D.C.K., Slack E., Hapfelmeier S., McCoy K.D., Macpherson A.J. Intestinal bacterial colonization induces mutualistic regulatory T-cell responses. *Immunity, 2011, Vol. 34, no. 5, pp. 794-806.*

10. Kullberg M.C., Jankovic D., Gorelick P.L., Caspar P., Letterio J.J., Cheever A.W., Sher A. Bacteria-triggered CD4(+) T regulatory cells suppress Helicobacter hepaticus-induced colitis. *J. Exp. Med., 2002, Vol. 196, no. 4, pp. 505-515.*

11. Kullberg M.C., Ward J.M., Gorelick P.L., Caspar P., Hieny S., Cheever A., Jankovic D., Sher A. Helicobacter hepaticus triggers colitis in specific-pathogen-free interleukin-10 (IL- 10)-deficient mice through an IL-12-and gamma interferon- dependent mechanism. *Infect. Immun., 1998, Vol. 66, no. 11, pp. 5157-5166.*

12. Lundgren A., Strömberg E., Sjöling A., Lindholm C., Enarsson K., Edebo A., Johnsson E., Suri-Payer E., Larsson P., Rudin A., Svennerholm A.-M., Lundin B. S. Mucosal FOXP3- expressing $CD4^{+}$ $CD25^{high}$ regulatory T-cells in Helicobacter pylori-infected patients. *Infect. Immun., 2005, Vol. 73, no. 1, pp. 523-531.*

13. Östman S., Rask C., Wold A.E., Hultkrantz S., Telemo E. Impaired regulatory T-cell function in germ-free mice. *Eur. J. Immunol., 2006, Vol. 36, no. 9, pp. 2336-2346.*

14. Round J.L., Mazmanian S.K. Inducible $Foxp3^{+}$ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc. Natl Acad. Sci. USA, 2010, Vol. 107, no. 27, pp. 12204-12209.*

15. Wenzel U.A., Magnusson M.K., Rydström A., Jonstrand C., Hengst J., Johansson M.E., Velcich A., Öhman L., Strid H., Sjövall H., Hansson G.C., Wick M.J. Spontaneous colitis in Muc2-deficient mice reflects clinical and cellular features of active ulcerative colitis. *PLoS One, 2014, Vol. 9, no. 6, e100217.* doi: 10.1371/journal.pone.0100217.

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