Errata

The committee has suggested an additional Errata chapter to address the issue of sections with alleged autoplagiaristic traits. This issue is regarding this thesis—*Modulation of the Mucosal and Systemic Immunity by Microbiota in Experimental Autoimmune Uveitis*—and the article—*Severity of Experimental Autoimmune Uveitis Is Reduced by Pretreatment with Live Probiotic Escherichia coli Nissle 1917*—I was a co-author of (Dusek et al., 2020). This chapter thus offers an additional citation of the article on pages 48, 49, 50, 51, 52, 90 and 91 where I used modified versions of the figures also published in the article (Dusek et al., 2020). Additionally, one paragraph and one figure legend on pages 38 and 49 were edited to meet optimal text standards. While the editing is usually minor, whole pages were included for lucidity.

Page 38

3. Materials and Methods

3.1.1. Animals

Initial experiment assessing immunomodulatory effects of E. coli in vivo was performed on 5 to 8 weeks old female C57BL/6J mice supplied to us by The Centre for Experimental Biomodels, First Faculty of Medicine, Charles University in Prague. Mice were housed at a conventional animal facility at the Department of Pharmacology, First Faculty of Medicine, Charles University in Prague. Mice with congenital defects were excluded from the study. Animals were distributed from a bulk randomly into cages.

Stimulation of bone marrow-derived macrophages, stimulation of bone marrow-derived dendritic cells and colonization experiment were performed on 10 weeks old female C57BL/6J mice obtained from the breeding colonies of the Institute of Microbiology of the Czech Academy of Sciences. All studies were carried out in accordance with the recommendations of the ethics standards defined by the EU legislation on the use of experimental animals (2010/63/EU) and the Czech animal welfare act. The protocols were approved by The Commission for AnimalWelfare of the First Faculty of Medicine of Charles University in Prague, The Ministry of Education, Youth and Sports (MSMT 9993/2017-2).

3.1.2. Experimental Autoimmune Uveitis Induction

EAU was induced by injection of interphotoreceptor retinoid-binding protein (IRBP) peptide in CFA containing heat-killed Mycobacterium tuberculosis H37Ra. This was immediately followed by application of pertussis toxin (PTx) (**Errata Table E1).**

Substance	Administration	Amount	Manufacturer
IRBP peptide 1—20 ([Homo sapiens] H2N-GPTHLFQPSLVLDMAKVLLD-OH)	SC	500 µg	New England Peptide, Gardner, MA, USA
CFA containing heat-killed *Mycobacterium tuberculosis* H37Ra	SC	3.3 mg/mL	Difco, Franklin Lakes, NJ, USA
Pertussis toxin	IP	1.2 µg	List Biological Laboratories, Inc., Campbell, CA, USA

Errata table E1. EAU induction details. Administration abbreviations: SC (subcutaneous), IP (intraperitoneal).

3.1.3. Probiotics

Escherichia coli Nissle 1917 (EcN; serotype O6:K5:H1; Ardeypharm GmbH, Herdecke, Germany) and Escherichia coli O83 (EcO; serotype O83:K24:H31; Dyntec spol. S.r.o., Terezin, Czech Republic) were cultured in Lennox´s version of Luria Bertani broth (Sigma-Aldrich, St. Louis, MO, USA) in a shaker incubator. Their optical densities (OD) were periodically measured by spectrophotometer at 600 nm (A600) to monitor population density. OD and volume was matched to cell count using previously established growth

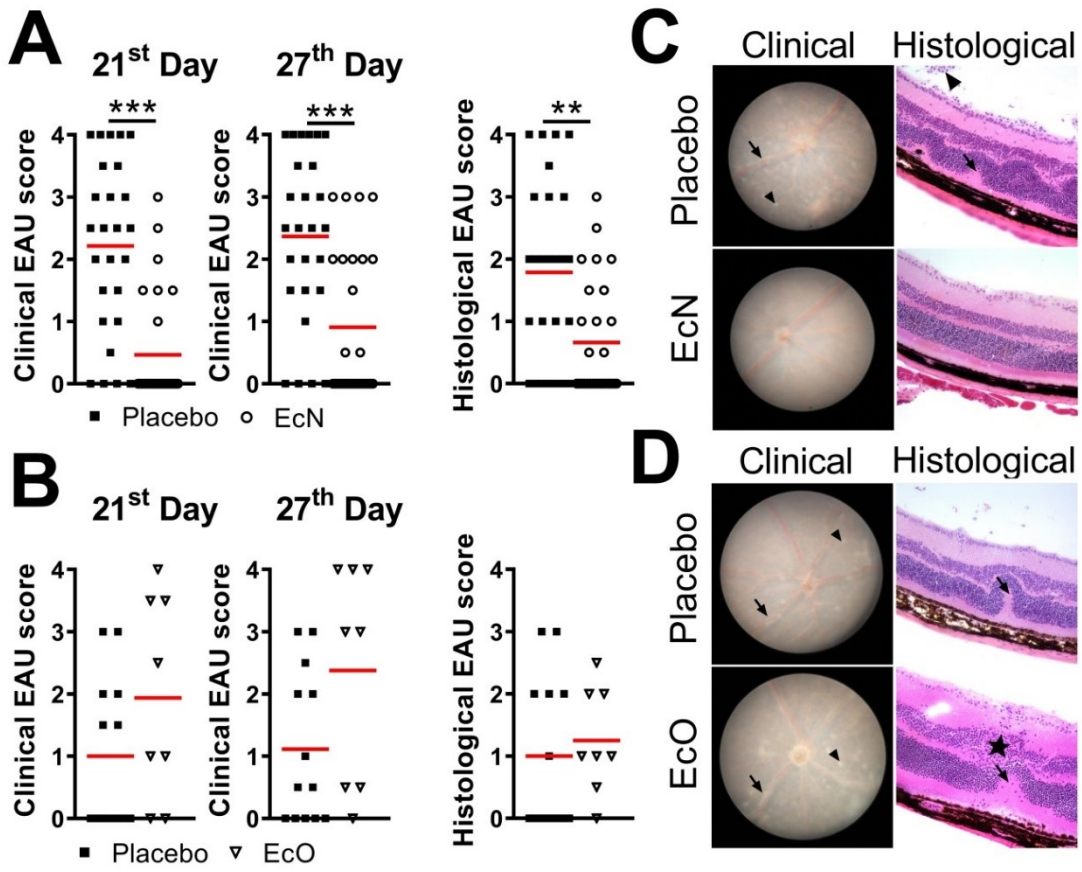

Figure 3. Treatment with live EcN reduces EAU severity while EcO treatment has no effect. Clinical score was assessed via in vivo fundus biomicroscopy—imaging of the posterior retina on the 21st and 27th day post-induction. Histology was performed on sections cut by cryostat and stained by hemotoxilin and eosin on the 28th day post-induction. Scores were evaluated on scale from 0 to 4 (4 being the most severe). Differences were quantified by unpaired Mann–Whitney test; graphs show individual values and red lines represent mean, ** $p < 0.01$ *** $p < 0.001$. Data are pools from 5 (EcN) or 2 (EcO) independent experiments. Figure shows representative pictures from prevention-and-treatment experiment schedule group. **(A)** and **(C)** Clinical and histological scores of EcN-treated mice showing reduced EAU scores [n = 26 (placebo), 28 (EcN) in total]. **(B)** and **(D)** Clinical and histological scores of EcO-treated mice showing no significant effect on EAU score [n = 13 (placebo), 8 (EcO) in total]. Grading evaluated EAU-associated pathologies. These include chorioretinal lesions (arrowhead), vascular sheathing (arrow) and optic nerve inflammation during clinical examination and infiltration of cells in the inner retina (star), cell infiltration of the vitreous body (vitritis, arrowhead) and retinal folds (arrow) during histological examination (optical magnification 200 ×). This figure was adopted from our published article and modified (Dusek et al., 2020).

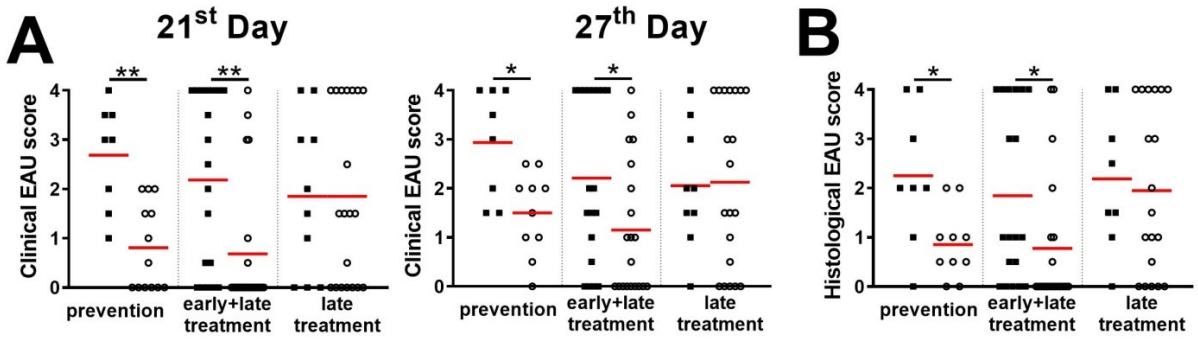

Figure 4. EcN treatment is only effective when given before or at the time of EAU induction. EAU severity was evaluated by **(A)** clinical score assessed by *in vivo* fundus biomicroscopy on the 21st and 27th day post-induction and by **(B)** histological score assessed by histological analysis at day 28 postinduction. Differences were quantified by unpaired Mann–Whitney test; * $p < 0.05$ ** $p < 0.01$; n = 8 (placebo prevention), 13 (EcN prevention), 10 (placebo late treatment), 20 (EcN late treatment), 19 (placebo early + late treatment) and 22 (EcN early + late treatment) in total. Graphs show individual values, open circles represent EAU severity scores of the placebo group, black squares represent EAU severity scores of EcN group, red lines represent mean. This figure was adopted from our published article and modified (Dusek et al., 2020).

Next, we focused on describing the immunological background of the process. Non-specific TCR stimulation response (anti-CD3/anti-CD28) of T cells derived from EcN-treaded mice, evaluated by production of pro-inflammatory cytokines, was unchanged (**Supplementary Figure S2**) and the proportions of Treg, Th17 and innate lymphoid cells 3 (ILC3) derived from mLN and cLN showed no differences among the groups either (**Supplementary Figure S3**). However, *ex vivo* stimulation of LNs with IRBP induced a lower expression of inflammatory cytokines by $CD4^+$ T cells in the EcN group compared to placebo (**Figure 5**). This reduced IRBP-specific T cells response occurred first in iLNs—at the site of immunisation on d7 (data not shown) after immunisation (before EAU manifestation) and propagated to other mLN and cLN later (d28). This suggests that at the time IRBP was presented, immunity was modulated and Th cells were primed to this specific antigen differently. This is in agreement with the timing of effective treatment which indicates that EcN affects antigen presentation of IRBP.

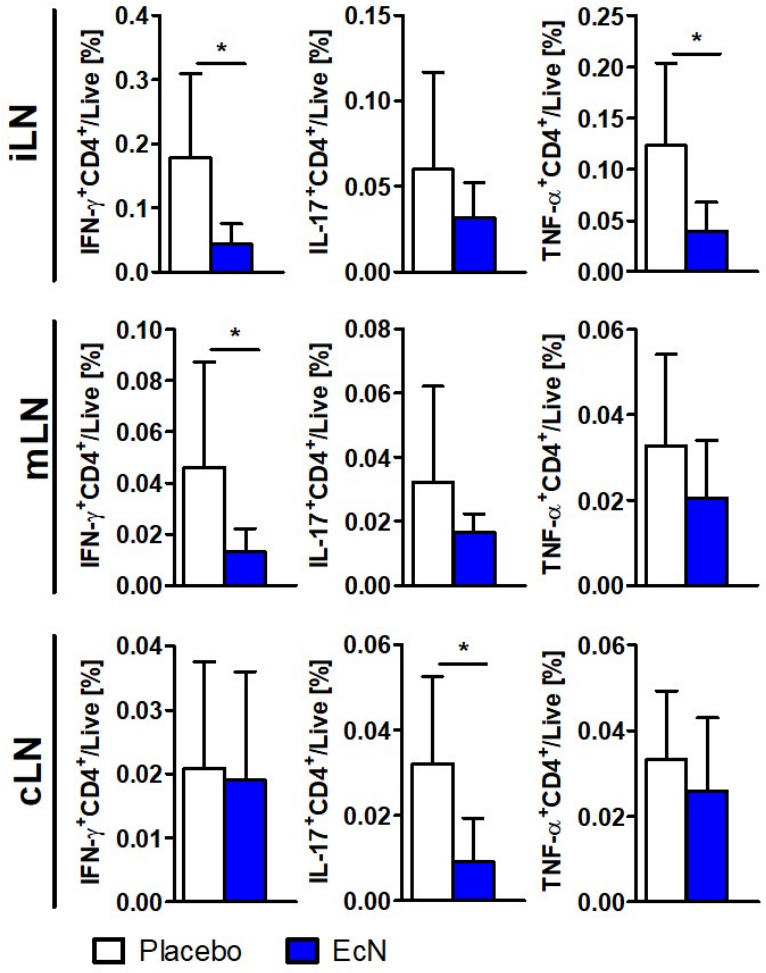

Figure 5. LN cells derived from EcN-treated mice are less responsive to IRBP stimulation. Specific response of inguinal, mesenteric and cervical lymph node-derived cells to IRBP was tested by a 40-hour incubation with 20 µg/mL of IRBP. Graphs show data from day 28 post-induction. Percentage of cytokine-producing populations was evaluated by immunophenotyping via flow cytometry. Differences were quantified by unpaired Mann Whitney test; * $p < 0.05$ (n = 5–8 per group). This figure was adopted from our published article and modified (Dusek et al., 2020).

To determine local gut mucosal immunity tuning we cultured PPs and assessed their cytokine production by ELISA. PPs derived from EcN-treated mice produced significantly lower amounts of inflammatory cytokines (TNF-α, IL-1β and IL-33) compared to placebo (**Figure 6**). Furthermore, EcN-treated mice harboured lower numbers of pro-inflammatory M1 macrophages ($CD38^+$, $Egr2^-$) in ileum (**Figure 7**) and a lower proportion of activated $iNOS^+$ macrophages in gut-draining mLNs compared to the placebo group (**Figure 8**). These data indicate that EcN modulates induction sites of the intestinal mucosal immunity to an anti-

inflammatory state and that macrophages could be involved in the EcN-induced immune tuning.

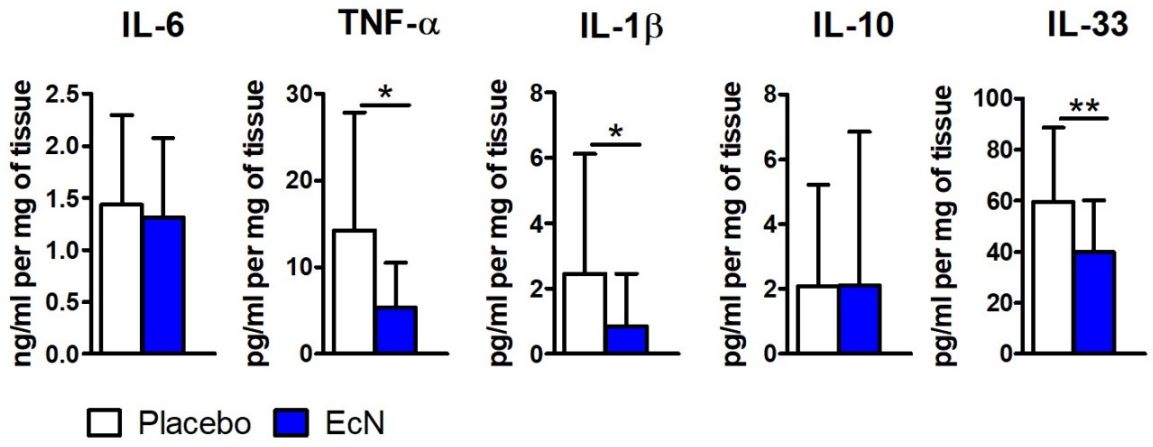

Figure 6. PPs derived from EcN-treated mice produce lower levels of inflammatory cytokines. PPs were collected at day 28 post-induction and cultured for 48h in a complete RPMI medium. Production of cytokines was evaluated by indirect ELISA. Data are pooled from 5 independent experiments (n = 25 per group in total) and differences were quantified by unpaired Mann–Whitney test; * $p < 0.05$ ** $p < 0.01$. This figure was adopted from our published article and modified (Dusek et al., 2020).

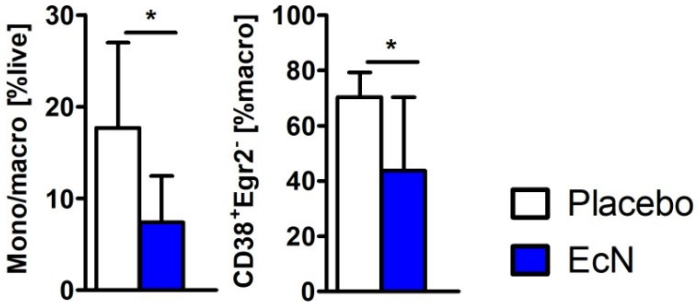

Figure 7. EcN treatment induces a decrease in macrophage populations in ileum. Tissue was collected at day 7 post-induction. Proportions of populations were assessed by FACS. Differences were quantified by unpaired Mann-Whitney test (n = 9 (placebo) or 7 (EcN)); *$p<0.05$, **$p<0.01$. Macrophages were defined as live $CD45^+F4/80^+CD11b^+$ cells and M1 ($CD38^+Egr2^+$). Markers were selected according to previously published data (Jablonski et al., 2015). This figure was adopted from our published article and modified (Dusek et al., 2020).

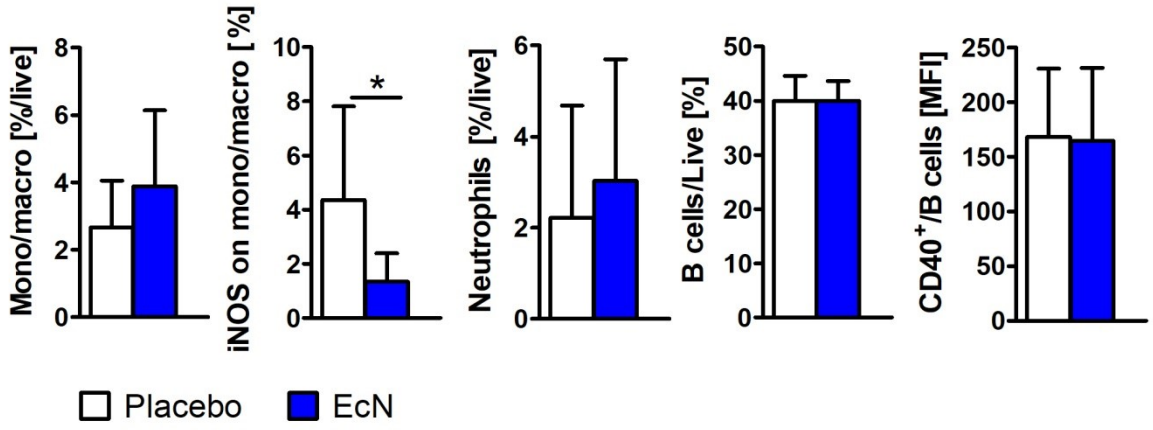

Figure 8. EcN treatment lowers the proportion of activated iNOS+ macrophages in mLN. The proportions of populations in mesenteric lymph nodes were assessed by FACS. Graphs show data from day 28 post-induction. Data are pooled from 2 independent experiments (n = 11 per group in total) and differences were quantified by unpaired Mann–Whitney test; * $p < 0.05$. The monocytes/macrophages are defined as live cells CD45+CD11c-B220-CD3-CD49b-Ly-6G-SSClo, neutrophils are defined as live cells CD45+CD11c-B220-CD3-CD49b-Ly-6G+ and B cells are described as live cells CD45+CD11c-B220+. This figure was adopted from our published article and modified (Dusek et al., 2020).

Collectively, the data from the initial in vivo experiments reveal several hallmarks suggesting the immune mechanism involved in treatment of EAU with *E coli*. First, only specific probiotics with immunomodulatory properties (EcN) are capable of reducing EAU severity. Second, EcN realizes its effects during the phase of Ag presentation and T cell priming. Third, the effects are not propagated through modulated proportions of Tregs, Th17 or ILC3 and fourth, immunomodulation is accompanied by an anti-inflammatory tuning at the induction sites of intestinal mucosal immunity with a shift in macrophage populations.

Based on the mentioned results we thus asked: What are the differences in immunomodulatory capacities responsible for the distinct effects of the microbes and how could the microbes affect the process of antigen presentation?

4.2. Colonization Experiments

4.2.1. *Theory and Experimental Design*

A simple explanation of the difference in immunomodulatory properties of the two microbes would be a distinct capacity to colonise the host´s gut. A long-term colonization

could provide a constant feed of immunomodulatory molecules to the host which may be crucial to effectively tune the immune system and decrease the inflammation.

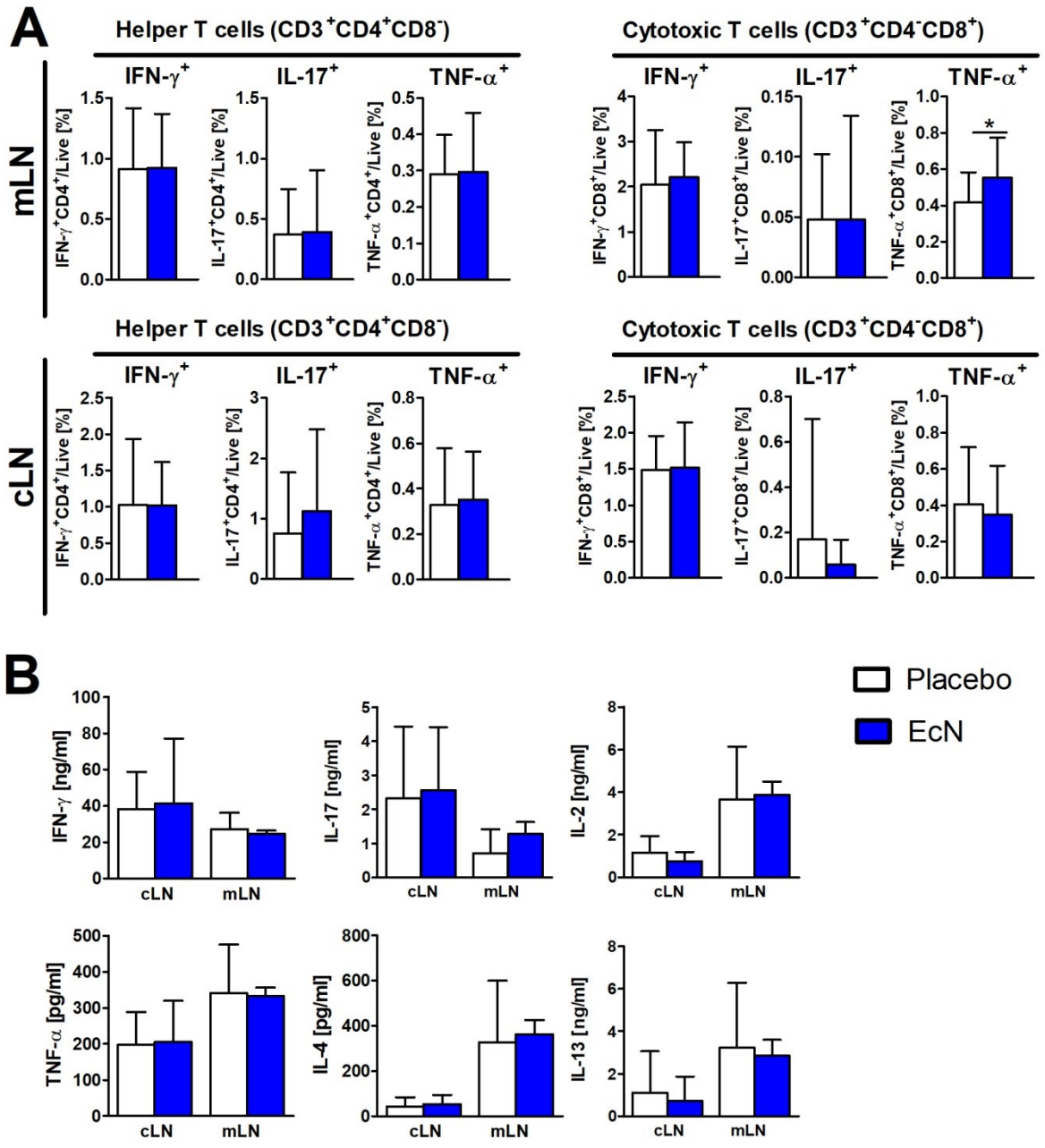

Figure S2. Responsiveness of T cells to anti-CD3/anti-CD28 stimulus remains unchanged by EcN treatment. Response was evaluated by intracellular expression of proinflammatory cytokines of T cells derived from cLNs and mLNs assessed by FACS (**A**) and production of cytokines by activated T cells derived from cLN and mLN assesed by ELISA (**B**). Data are pools from 5 independent experiments (n = 24 per group in total). Differences were quantified by unpaired Mann–Whitney test; *p<0.05. Data are from day 28 post-induction. This figure was adopted from our published article and modified (Dusek et al., 2020).

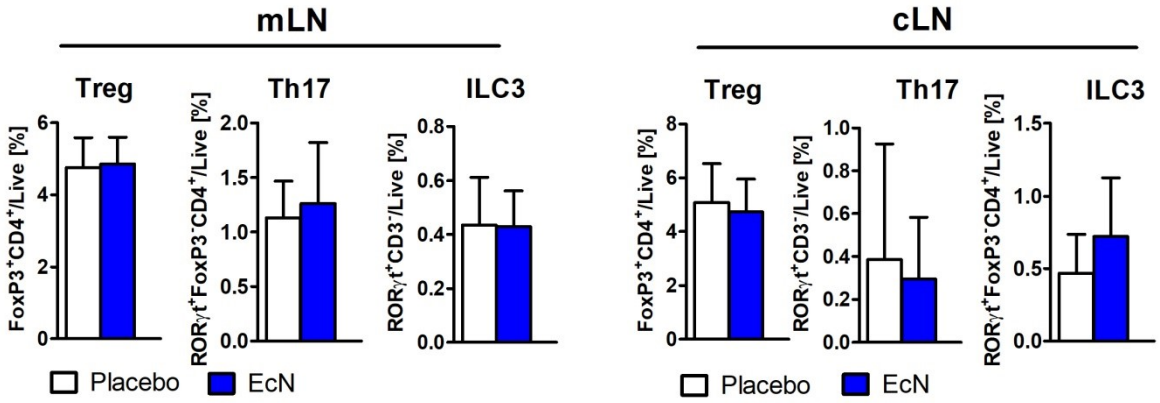

Figure S3. Treatment of EAU with EcN does not affect proportions of Treg, Th17 and ILC3 populations in mLNs or cLNs. Proportions of cells in mesenteric and cervical lymph nodes were evaluated by FACS at day 28 post-induction (n = 22 (placebo), 23 (EcN) in total). This figure was adopted from our published article and modified (Dusek et al., 2020).

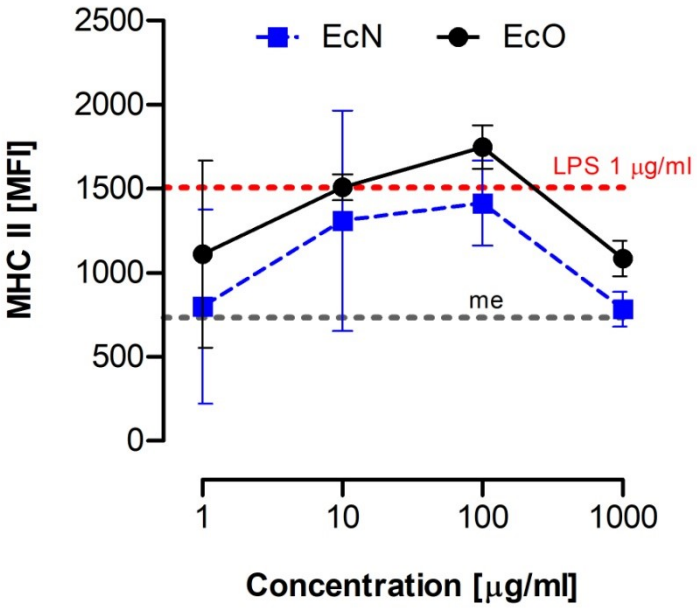

Figure S4. There are no significant differences in the expression of MHC II by BMDM in reaction to the two lysates. Data were obtained by FACS, gated to singlet, live, F4/80 positive cells and adjusted to mean fluorescence intensity (MFI) of each sample. X axis shows concentration of the stimulus—the bacterial lysate. Red dotted line represents positive control — 1 µg/ml of LPS, black dotted line represents negative control—culture medium only. Figure shows representative graph from 1 out of 3 independent experiments. Differences were quantified by One-way ANOVA with Tukey´s multiple comparison test; * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.