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# Menthol pretreatment alleviates *Campylobacter jejuni* induced enterocolitis in human gut microbiota-associated IL-10$^{-/-}$ mice

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**Supplementary Table 1. Clinical scores (maximum 12 points).**

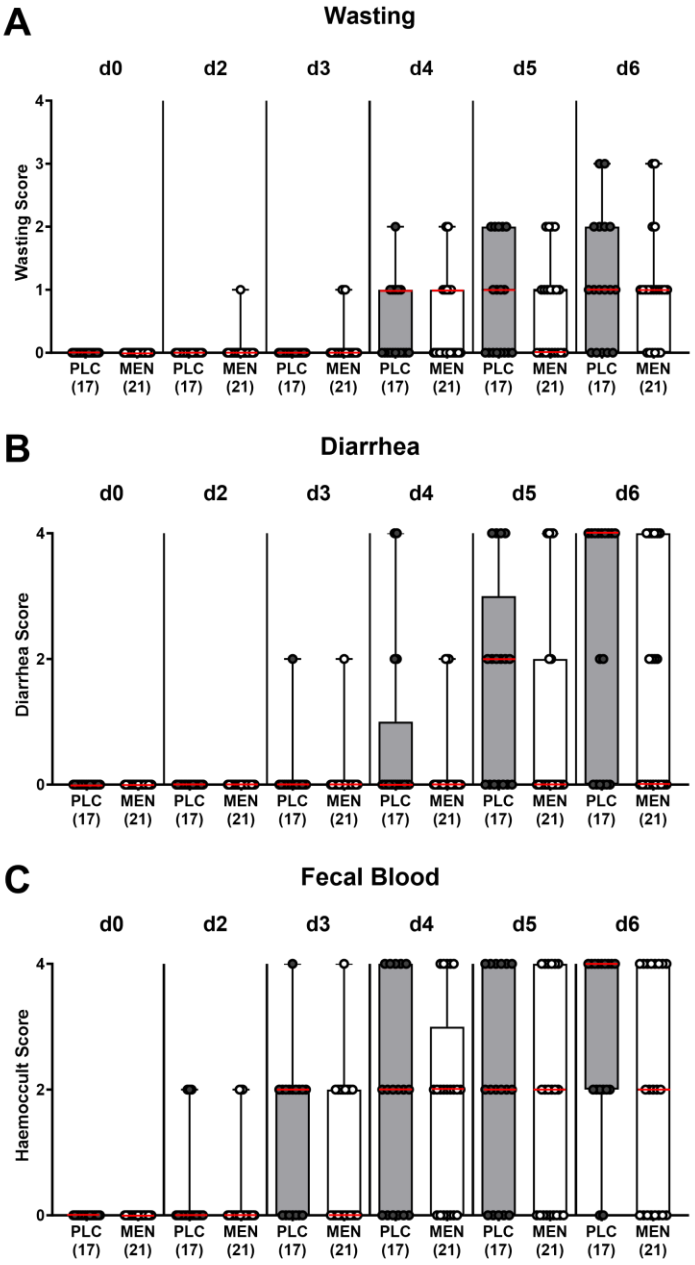
| Clinical aspect | Scores |
|---|---|
| **Wasting symptoms** | 0: normal<br>1: ruffled fur<br>2: less locomotion<br>3: isolation<br>4: severely compromised locomotion, pre-final aspect |
| **Stool consistency** | 0: formed feces<br>2: pasty feces<br>4: liquid feces |
| **Fecal blood** | 0: no blood<br>2: microscopic detection of blood by the Guajac method using Haemoccult, Beckman Coulter/PCD, Germany<br>4: macroscopic blood visible |

**Supplementary Table 2: Histopathological scores (maximum 4 points).**

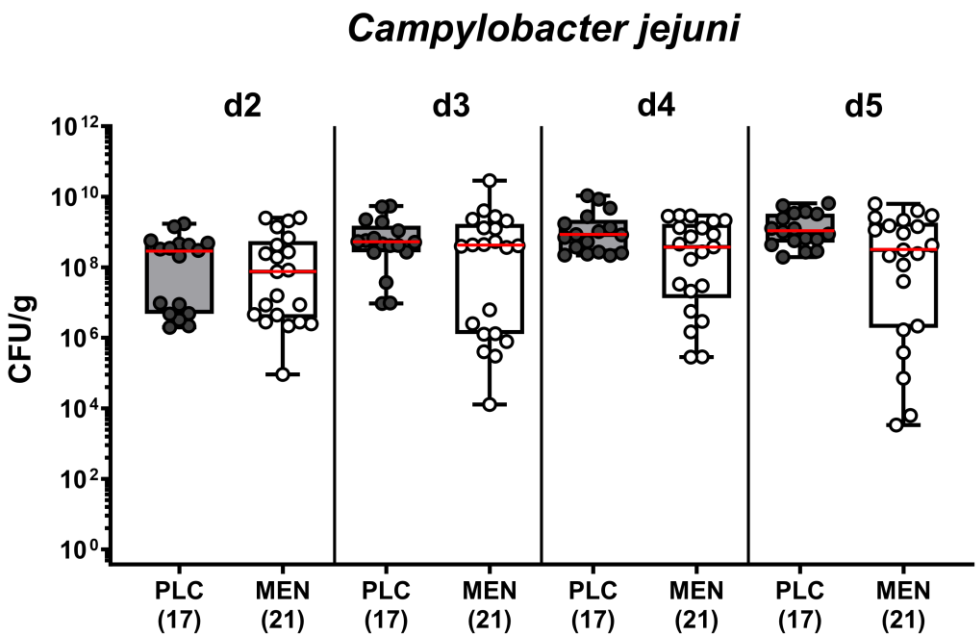
| | |
|---|---|
| **Score 0** | Normal epithelium without inflammatory cell infiltrates. |
| **Score 1** | Minimal inflammatory cell infiltrates in the mucosa with intact epithelium. |
| **Score 2** | Mild inflammatory cell infiltrates in the mucosa and submucosa with mild hyperplasia and mild goblet cell loss. |
| **Score 3** | Moderate inflammatory cell infiltrates in the mucosa with moderate goblet cell loss. |
| **Score 4** | Marked inflammatory cell infiltration into the mucosa and submucosa with marked goblet cell loss, multiple crypt abscesses, and crypt loss. |

**Supplementary Table 3: Primary antibodies for *in situ* immunohistochemical analyses**

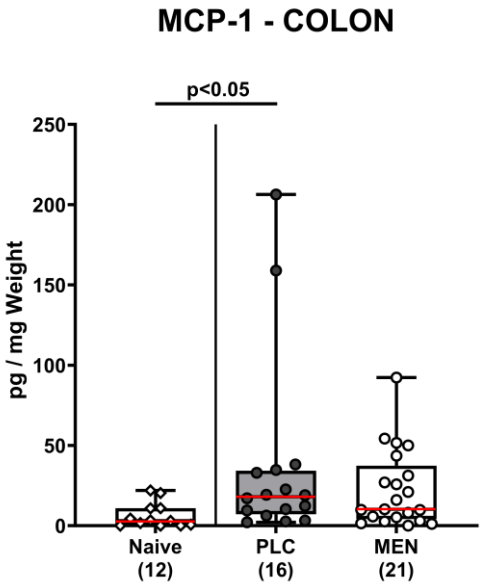
| Cells | Primary antibody |
|---|---|
| Apoptotic epithelial cells | cleaved caspase-3 (Asp175, Cell Signaling, Beverly, MA, USA, 1:200) |
| Macrophages/monocytes | F4/80 (no. 14-4801, clone BM8, eBioscience, San Diego, CA, USA, 1:50 |
| Neutrophils | MPO7 (No. A0398, Dako, Glostrup, Denmark, 1:500) |
| T lymphocytes | CD3 (no. N1580, Dako, 1:10) |
| B lymphocytes | B220 (no. 14-0452-81, eBioscience, San Diego, CA, USA;1:200). |




**Supplementary Figure S1. Clinical signs of campylobacteriosis over time following *C. jejuni* infection of hma IL-10$^{-/-}$ mice with menthol pre-treatment.** Hma IL-10$^{-/-}$ mice were orally pretreated with menthol (MEN, white bars) or placebo (PLC, grey bars) and infected with *C. jejuni* on day 0 (d0) and d1 by gavage. The clinical signs of campylobacteriosis such as **(A)** wasting symptoms, **(B)** diarrhea, and **(C)** fecal blood were recorded with individual scores (see methods). Box plots (25$^{th}$ and 75$^{th}$ percentiles), whiskers (minimum and maximum values), medians (red bar in boxes), and numbers of analyzed mice (in parentheses) from three experiments are given.




**Supplementary Figure S2. Pathogen numbers in fecal samples taken following *C. jejuni* infection of hma IL-10$^{-/-}$ mice with menthol pre-treatment.** Hma IL-10$^{-/-}$ mice were orally pretreated with menthol (MEN, white bars) or placebo (PLC, grey bars) and infected with *C. jejuni* on day 0 (d0) and d1 by gavage. The fecal C. jejuni numbers were determined at defined time points post-infection by culture. Box plots (25$^{th}$ and 75$^{th}$ percentiles), whiskers (minimum and maximum values), medians (red bar in boxes), and numbers of analyzed mice (in parentheses) from three experiments are given. CFU, colony-forming units.




**Supplementary Figure S3. Colonic MCP-1 secretion in *C. jejuni* infected hma IL-10$^{-/-}$ mice with menthol pretreatment.** Hma IL-10$^{-/-}$ mice were orally pretreated with menthol (MEN, white bars) or placebo (PLC, grey bars) and infected with *C. jejuni* on days 0 and 1 by gavage. On day 6 post-infection, MCP-1 concentrations were measured in *ex vivo* biopsies sampled from the colon. Naive mice (non-infected without pretreatment) served as negative controls. Box plots (25$^{th}$ and 75$^{th}$ percentiles), whiskers (minimum and maximum values), medians (red bar in boxes), significance levels (p values) determined by the Kruskal-Wallis test with Dunn's post hoc test, and numbers of analyzed mice (in parentheses) from three experiments are given. The Grubb's test was used to identify definite outliers.