Table S1. Oligonucleotides sequences (5’-3’) for real-time quantitative PCR analysis

|  |  |  |
| --- | --- | --- |
| Gene symbol | Forward sequences | Reverse sequences |
| Total bacteria (*16S rDNA*) | ACTCCTACGGGAGGCAGCAGT | ATTACCGCGGCTGCTGGC |
| *Reg3β* | GGCTTCATTCTTGTCCTCCA | TCCACCTCCATTGGGTTCT |
| *Reg3γ* | AAGCTTCCTTCCTGTCCTCC | TCCACCTCTGTTGGGTTCAT |
| *MUC2* | GCTGACGAGTGGTTGGTGAATG | GATGAGGTGGCAGACAGGAGAC |
| *IL-1β* | CCAGCTTCAAATCTCACAGCAG | CTTCTTTGGGTATTGCTTGGGATC |
| *IL-18* | CAGGCCTGACATCTTCTGCAA | TCTGACATGGCAGCCATTGT |
| *TNFα* | CACAGAAAGCATGATCCGCGACGT | CGGCAGAGAGGAGGTTGACTTTCT |
| *Beta-actin* | CTGTCCCTGTATGCCTCTG | ATGTCACGCACGATTTCC |

Reg3β, regenerating islet-derived 3 family of proteins beta; Reg3γ, regenerating islet-derived 3 family of proteins gamma; MUC2, mucin 2; IL-1β, interleukin-1 beta; IL-18, interleukin-18; TNFα, tumor necrosis factor alpha.

Table S2. Concentrations of cytokines/chemokines in the colon tissue 7 days after *Citrobacter rodentium* infection

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Cytokines/chemokines | CD(pg/mL) |  | CS(pg/mL) |  | CE(pg/mL) |  | *P* value |
|  |  mean |  SEM |  |  mean |  SEM |  |  mean |  SEM |  |  |
| IL-7 | 8.88 | 1.61 |  | 4.31 | 0.79 |  | 5.81 | 2.07 |  | 0.100 |
| IP-10 | 39.62 | 10.28 |  | 25.50 | 6.26 |  | 14.02 | 7.61 |  | 0.111 |
| G-CSF | 18.67 | 5.70 |  | 6.22 | 1.11 |  | 6.61 | 2.26 |  | 0.107 |
| IL-1β | 19.56 | 1.94 |  | 17.58 | 3.21 |  | 13.65 | 0.91 |  | 0.171 |
| MIG | 215.90 | 78.57 |  | 191.00 | 68.11 |  | 42.19 | 18.48 |  | 0.139 |
| GM-CSF | 8.78 | 1.30 |  | 9.82 | 1.11 |  | 12.76 | 2.75 |  | 0.680 |
| IL-17 | 5.56 | 3.32 |  | 1.75 | 0.67 |  | 1.37 | 0.65 |  | 0.275 |
| IFNγ | 6.19 | 2.03 |  | 10.11 | 2.42 |  | 11.54 | 4.67 |  | 0.717 |
| IL-1α | 58.52 | 8.93 |  | 37.67 | 6.23 |  | 43.01 | 9.36 |  | 0.227 |
| IL-3 | 0.48 | 0.12 |  | 1.62 | 0.60 |  | 1.13 | 0.40 |  | 0.423 |
| IL-4 | 0.42 | 0.07 |  | 0.32 | 0.03 |  | 0.33 | 0.03 |  | 0.153 |
| IL-6 | 17.15 | 8.64 |  | 5.25 | 1.92 |  | 6.62 | 2.01 |  | 0.627 |
| IL-10 | 14.90 | 4.16 |  | 14.66 | 4.14 |  | 14.14 | 3.05 |  | 0.990 |
| IL-12p40 | 5.49 | 0.67 |  | 8.70 | 2.54 |  | 13.83 | 4.28 |  | 0.381 |
| IL-12p70 | 6.46 | 1.64 |  | 5.98 | 1.73 |  | 8.02 | 2.60 |  | 0.770 |
| IL-15 | 26.19 | 4.49 |  | 21.64 | 1.63 |  | 21.13 | 2.84 |  | 0.461 |
| KC | 66.46 | 22.51 |  | 20.36 | 5.60 |  | 20.41 | 7.75 |  | 0.235 |
| LIF | 20.62 | 7.87 |  | 10.84 | 2.05 |  | 13.76 | 4.10 |  | 0.858 |
| M-CSF | 3.80 | 0.69 |  | 3.71 | 0.66 |  | 3.91 | 0.69 |  | 0.979 |
| MIP-1α | 28.11 | 8.41 |  | 12.63 | 1.63 |  | 17.49 | 4.14 |  | 0.592 |
| TNFα | 2.76 | 0.55 |  | 2.45 | 0.35 |  | 2.91 | 0.84 |  | 0.862 |
| VEGF | 2.39 | 0.41 |  | 1.61 | 0.40 |  | 1.56 | 0.35 |  | 0.271 |

CD, choline deficient; CS, choline sufficient; CE, choline excess; IL-7, interleukin 7; IP-10, IFN-γ-inducible protein 10, CXCL10; G-CSF, granulocyte-colony stimulating factor; IL-1β, interleukin-1 beta; MIG, monokine induced by gamma interferon, CXCL9; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-17, interleukin 17; IFNγ, interferon gamma; IL-1α, interleukin-1 alpha; IL-3, interleukin 3; IL-4, interleukin 4; IL-6, interleukin 6; IL-10, interleukin 10; IL-12p40, interleukin 12 p40; IL-12p70, interleukin 12 p70; IL-15, interleukin 15; KC, keratinocyte chemoattractant, CXCL1; LIF, leukemia inhibitory factor; M-CSF, macrophage colony-stimulating factor, CSF1; MIP-1α, macrophage inflammatory protein 1-alpha, CCL3; TNFα, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

Data are presented as mean ± SEM (n = 10). The lower detection limit for listed cytokines is 0.64 pg/mL.

Table S3. Concentrations of cytokines/chemokines in the colon tissue 4 weeks after dietary treatment

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Cytokines/chemokines | CD(pg/mL) |  | CS(pg/mL) |  | CE(pg/mL) |  | *P* value |
|  |  mean |  SEM |  |  mean |  SEM |  |  mean |  SEM |  |  |
| MCP-1 | 5.45 | 2.03 |  | 5.98 | 0.85 |  | 5.93 | 0.63 |  | 0.051 |
| MIP-2 | 1.49 | 0.22 |  | 2.86 | 0.45 |  | 2.18 | 0.49 |  | 0.059 |
| IFNγ | 0.57 | 0.14 |  | 0.22 | 0.04 |  | 0.41 | 0.09 |  | 0.073 |
| IP-10 | 4.80 | 2.59 |  | 21.35 | 10.57 |  | 6.80 | 5.31 |  | 0.302 |
| IL-1β | 11.37 | 4.05 |  | 5.58 | 0.97 |  | 6.48 | 2.10 |  | 0.533 |
| GM-CSF | 0.47 | 0.08 |  | 0.40 | 0.06 |  | 0.48 | 0.06 |  | 0.688 |
| IL-17 | 0.03 | 0.01 |  | 0.08 | 0.03 |  | 0.07 | 0.02 |  | 0.106 |
| IL-4 | 0.11 | 0.05 |  | 0.06 | 0.01 |  | 0.22 | 0.07 |  | 0.309 |
| KC | 2.16 | 1.09 |  | 5.84 | 1.72 |  | 2.89 | 2.35 |  | 0.296 |
| MIP-1α | 5.88 | 1.13 |  | 8.47 | 0.71 |  | 7.39 | 1.00 |  | 0.209 |
| TNFα | 2.34 | 0.64 |  | 2.43 | 0.22 |  | 2.97 | 0.29 |  | 0.208 |
| IL-22 | 0.16 | 0.05 |  | 0.27 | 0.10 |  | 0.31 | 0.10 |  | 0.374 |
| MMP-9 | 1901.00 | 247.80 |  | 2136.00 | 228.10 |  | 2091.00 | 365.30 |  | 0.811 |

CD, choline deficient; CS, choline sufficient; CE, choline excess; MCP-1, monocyte chemoattractant protein-1; MIP-2, macrophage inflammatory protein 2; IFNγ, interferon gamma; IP-10, IFN-γ-inducible protein 10; IL-1β, interleukin-1 beta; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-17, interleukin 17; IL-4, interleukin 4; KC, keratinocyte chemoattractant, CXCL1; MIP-1α, macrophage inflammatory protein-1 alpha; TNFα, tumor necrosis factor alpha; IL-22, interleukin 22; MMP-9, matrix metalloproteinase-9. Data are presented as mean ± SEM. CD, n = 8; CS and CE, n = 6.

**Supplementary Figures**

****

Figure S1. (a) Colonic PC and (b) PE concentration without *Citrobacter rodentium* infection in choline-deficient (CD), choline-sufficient (CS), and choline-excess (CE) mice. For all treatment groups, n = 8. (c) Colonic PE concentrations 7 days after *C. rodentium* infection in CD, CS, and CE mice. n = 10. (d) Mouse body weight during dietary treatment for three weeks before *C. rodentium* infection. n = 10. Data are presented as mean ± SEM. Each dot represents an individual mouse.



Figure S2. The mRNA expression level of (a) interleukin-1 beta (*IL-1β*), (b) interleukin-18 (IL-18), and (c) tumor necrosis factor alpha (*TNFα*) in the colon from choline-deficient (CD), choline-sufficient (CS), and choline-excess (CE) mice four weeks after dietary treatment. n = 8. Data are presented as mean ± SEM. (d) Total bacterial load in feces from CD, CS, and CE mice four weeks after dietary treatment. n = 8. Data are presented as mean ± SEM. Each dot represents an individual mouse.



Figure S3. LEfSe analysis showed differentially abundant bacterial genera that varied by choline treatments. CD, choline deficient; CS, choline sufficient; CE, choline excess. Significance of differences was determined using Kruskal-Wallis test (*P* < 0.05) with a linear discriminant analysis (LDA) score (log10) > 2. For all treatment groups, n = 8.