Communication between CNS and gut microbiota in a viral model for multiple sclerosis

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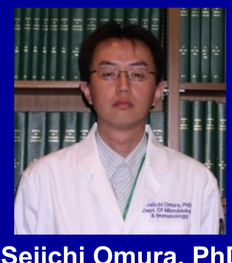

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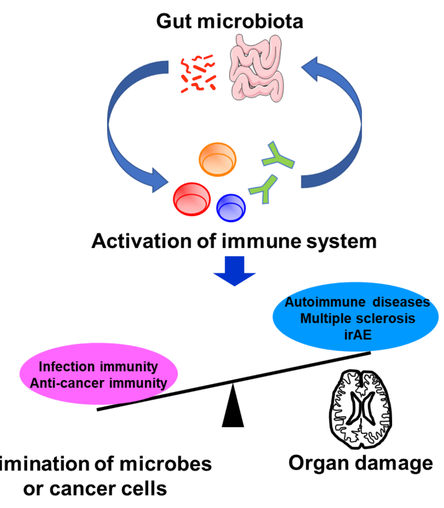
Abstract

While the immune system eliminates microbes and cancer, playing protective roles, uncontrolled immune responses are detrimental, causing immune-mediated tissue damage (immunopathology), i.e. an immune-related adverse event (irAE) by ant-cancer immune checkpoint inhibitors. Gut microbiota can affect systemic immune responses, contributing to pathology in remote organs including the central nervous system (CNS), positively or negatively. Multiple sclerosis (MS) is an inflammatory demyelinating disease in the CNS, whose pathogenesis has also been linked to altered microbiota. We aim to clarify how gut microbiota, the immune system, and the CNS communicate one another using an animal model for MS, Theiler's murine encephalomyelitis virus (TMEV) infection. We infected mice with TMEV and harvested the CNS tissues and feces on 4, 7, and 35 days post infection (p.i.); TMEV induces CNS inflammation about 1 month p.i. We examined the CNS transcriptome by RNA sequencing, and fecal microbiota by 16S rRNA amplicon sequencing. The diversity of microbiota 35 days p.i. was higher than those from the other time points as well as uninfected control samples. Following the CNS disease onset, 35 days p.i., while increased *Marvinbryantia* and *Coprococcus* genera correlated with upregulation of acquired immune response genes, particularly immunoglobulin (Ig) genes in the CNS, relative abundance of each of the bacterial genera correlated with a different set of Ig genes. Using a principal component analysis (PCA), we identified decreased *Anaeroplasma* genus and increased genera of the family *S24-7* were correlated with principal component (PC) 2 values that reflect CNS inflammation. Therefore, CNS virus infection could change the gut microbiota that skews the immune responses toward pro-inflammatory, resulting in CNS inflammation. Microbiota can alter metabolome including short-chain fatty acid metabolism that contributes to pro-inflammatory immune responses; such chemical changes may be involved in the gut–immunity–brain communication.

Introduction

- Communications between gut microbiota and immune system activate systemic immune responses that eliminate microbes and cancer.
- Uncontrolled excessive immune responses cause immune-mediated tissue damage, immunopathology.
- Immunopathology: autoimmune diseases, multiple sclerosis (MS), and immune-related adverse event (irAE) by anti-cancer immune checkpoint inhibitors (ICIs).

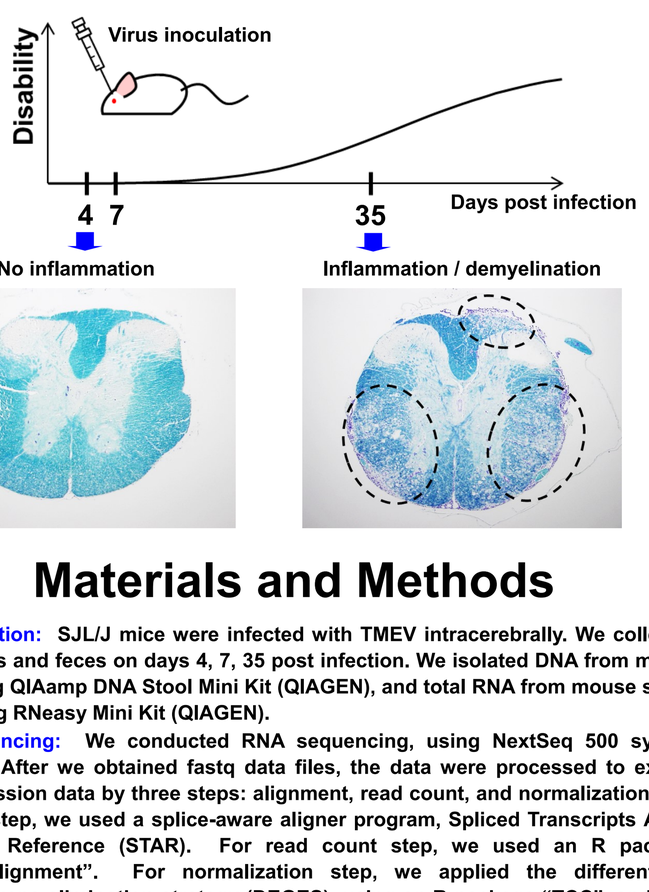


Hypothesis

Communication failure between gut microbiota, immune system, and distant organ induces immune-mediated diseases

Multiple sclerosis (MS) and its animal model

- MS is a chronic immune-mediated disease in the central nervous system (CNS), characterized by inflammation and demyelination (loss of myelin).
- Gut microbiota changes have been reported in MS.
- CNS infection of Theiler's murine encephalomyelitis virus (TMEV) in mice induces an MS-like CNS disease; an animal model for MS.
- CNS immune cell infiltration starts 1 week after CNS TMEV inoculation, while MS-like demyelination becomes obvious 1 month after infection.



Materials and Methods

TMEV infection: SJL/J mice were infected with TMEV intracerebrally. We collected spinal cords and feces on days 4, 7, 35 post infection. We isolated DNA from mouse feces, using QIAamp DNA Stool Mini Kit (QIAGEN), and total RNA from mouse spinal cords, using RNeasy Mini Kit (QIAGEN).

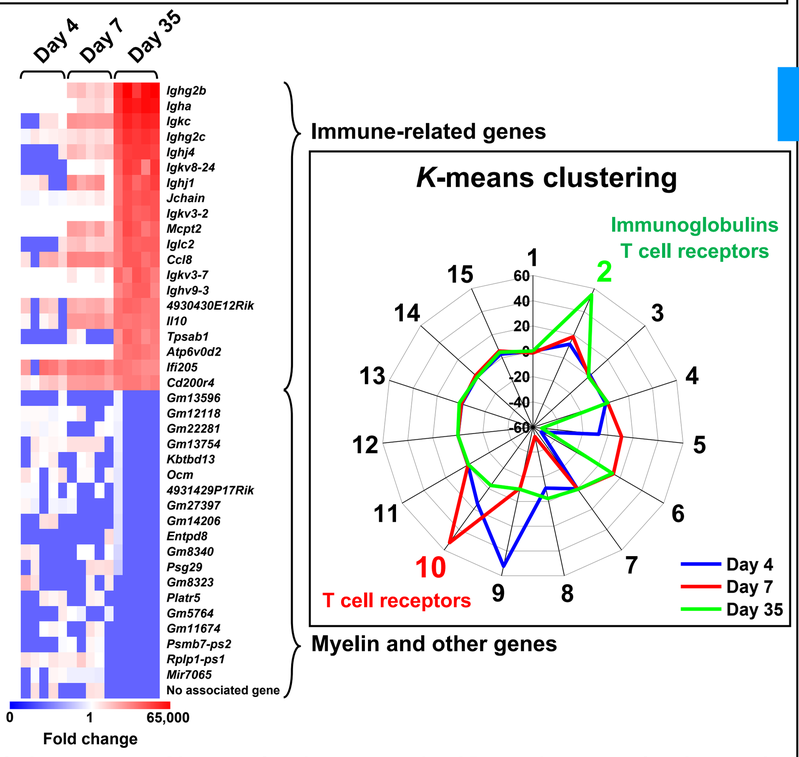
RNA sequencing: We conducted RNA sequencing, using NextSeq 500 system (Illumina). After we obtained fastq data files, the data were processed to extract gene expression data by three steps: alignment, read count, and normalization. For alignment step, we used a splice-aware aligner program, Spliced Transcripts Alignment to a Reference (STAR). For read count step, we used an R package "GenomicAlignment". For normalization step, we applied the differentially-expressed gene elimination strategy (DEGES), using an R package "TCC", and read count per kilobase (RPK).

16S rRNA amplicon sequencing: We conducted 16S rRNA amplicon sequencing, using MiSeq (Illumina). After we obtained fastq data files, the data were analyzed using QIIME2.

Bioinformatics: Heat map was drawn, using R packages "genefilter" and "gplots". *K*-means clustering was conducted, using an R package "cclust". Principal component analysis (PCA) was conducted, using an R program "prcomp". Pattern matching was conducted, using R.

CNS transcriptome

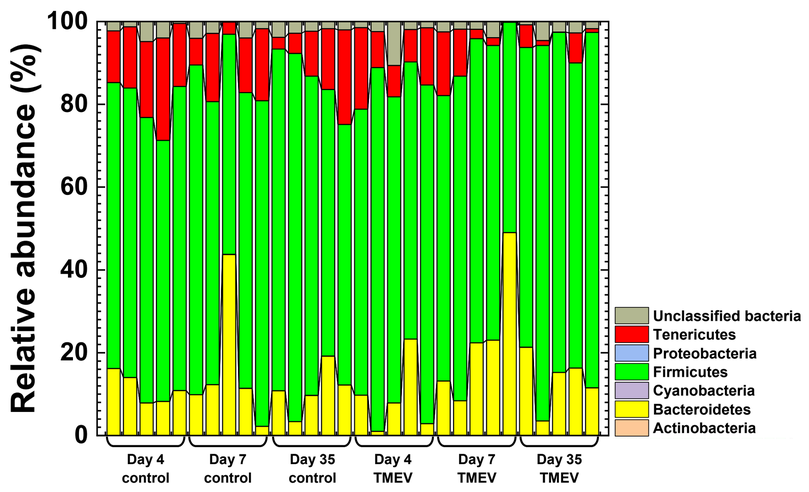
In the CNS, upregulation of acquired immune response (T cell and antibody) genes starts on day 7



- In heatmap, top 20 upregulated genes on day 35 were immune-related genes including immunoglobulin (Ig) genes that started upregulation on day 7.
- In radar chart based on *k*-means clustering, cluster 2 included acquired immune response genes upregulated over the time course: Ig and T cell receptor genes.
- Cluster 10 includes a set of T cell receptor genes only upregulated on day 7.
- On day 4, innate immune response genes were upregulated, including genes associated with inflammasome, i.e. NLR family, pyrin domain containing 3 (*Nlrp3*).

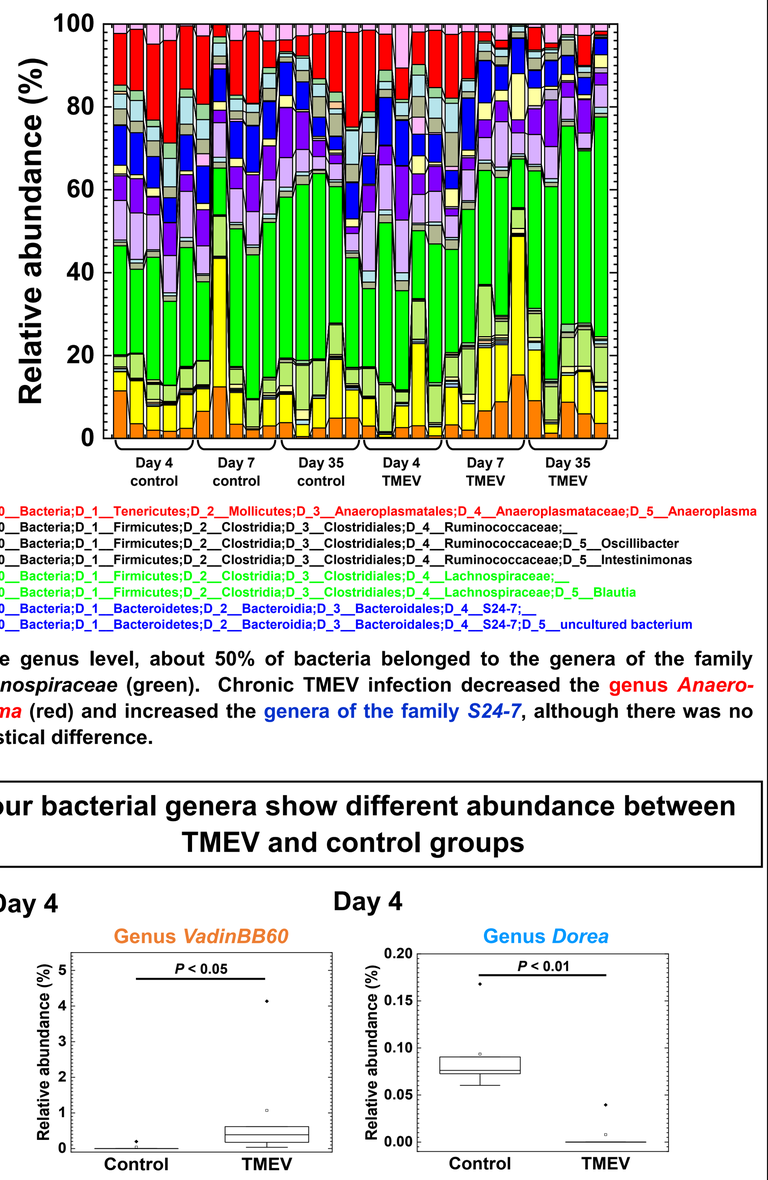
Fecal microbiome

In the phylum level, the phylum *Tenericutes* is decreased on day 35 of TMEV infection



Cumulative bar plot showed the relative abundance of bacterial phyla in feces. Most bacteria belonged to the phylum *Firmicutes* (green). The other bacteria were classified into the phyla *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Proteobacteria*, or *Tenericutes*. On day 35 of TMEV infection, the phylum *Tenericutes* (red), composed of only the genus *Anaeroplasma*, tended to be decreased, although there was no statistical difference compared with controls.

In the genus level, chronic TMEV infection decreases the genus *Anaeroplasma* and increases the genera of family *S24-7*

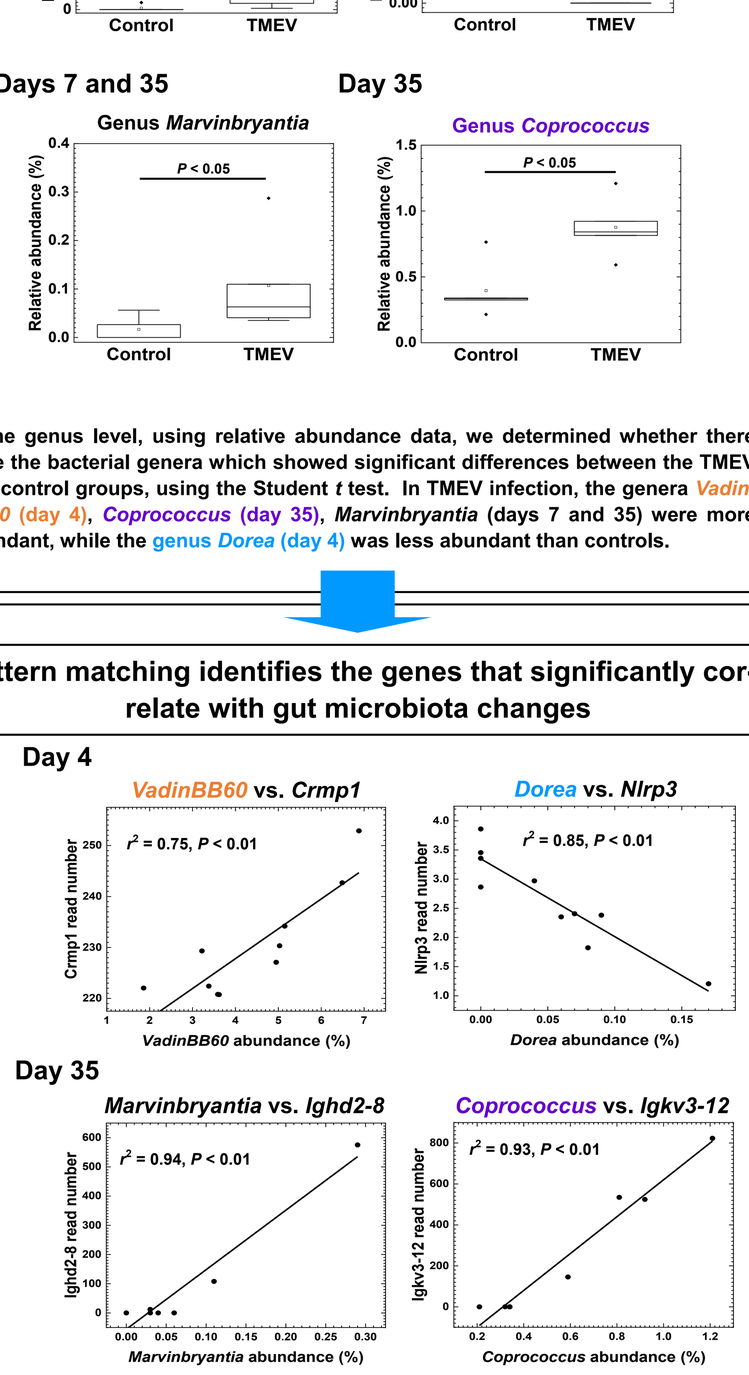


In the genus level, about 50% of bacteria belonged to the genera of the family *Lachnospiraceae* (green). Chronic TMEV infection decreased the genus *Anaeroplasma* (red) and increased the genera of the family *S24-7*, although there was no statistical difference.

Four bacterial genera show different abundance between TMEV and control groups

In the genus level, using relative abundance data, we determined whether there were the bacterial genera which showed significant differences between the TMEV and control groups, using the Student *t* test. In TMEV infection, the genera *VadinBB60* (day 4), *Coprococcus* (day 35), *Marvinbryantia* (days 7 and 35) were more abundant, while the genus *Dorea* (day 4) was less abundant than controls.

Pattern matching identifies the genes that significantly correlate with gut microbiota changes

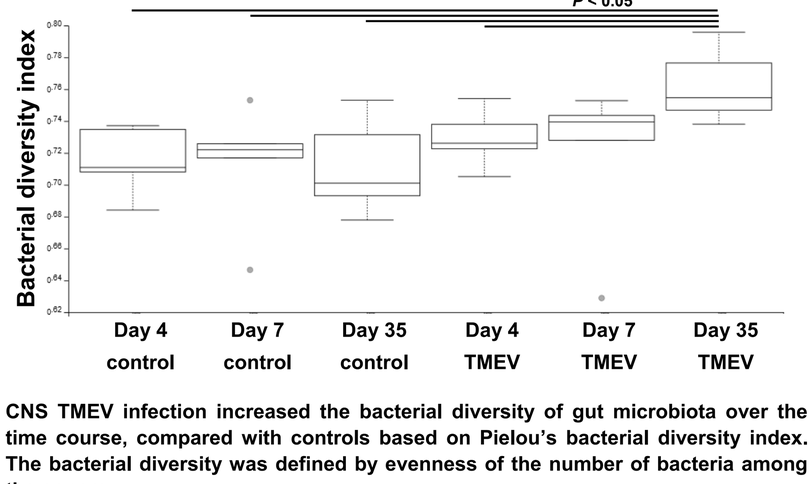


To identify the genes which reflect the changes in fecal microbiota, we conducted pattern matching between the CNS transcriptome and relative abundance of the four significantly altered bacterial genera following TMEV infection.

- On day 4, an increase of the genus *VadinBB60* correlated with 90 genes, including neuron-related genes, collapsin response mediator protein 1 (*Crmp1*), while a decrease of the genus *Dorea* correlated with 204 genes ($r^2 > 0.64$), including innate immune response genes, *Nlrp3.*
- On day 35, the genera *Marvinbryantia* and *Coprococcus* correlated with 586 and 3505 genes, respectively, both of which include immunoglobulin (Ig) genes.
- The genera *Marvinbryantia* and *Coprococcus* correlated with distinct Ig genes.

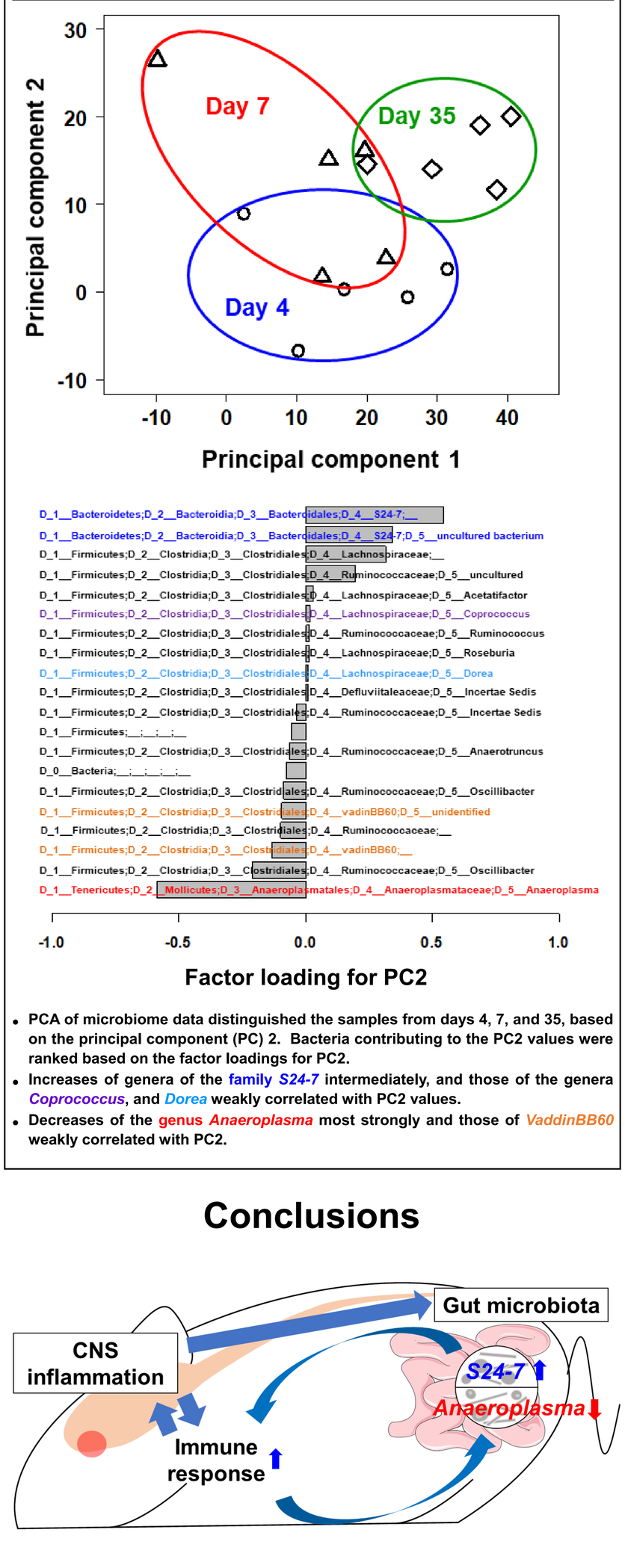
Results

Bacterial diversity of fecal microbiome is increased in TMEV-infected mice on day 35



CNS TMEV infection increased the bacterial diversity of gut microbiota over the time course, compared with controls based on Pielou's bacterial diversity index. The bacterial diversity was defined by evenness of the number of bacteria among the genera.

Principal component analysis (PCA) distinguishes overall microbiome patterns depending on the time points



- PCA of microbiome data distinguished the samples from days 4, 7, and 35, based on the principal component (PC) 2. Bacteria contributing to the PC2 values were ranked based on the factor loadings for PC2.
- Increases of genera of the family *S24-7* intermediately, and those of the genera *Coprococcus*, and *Dorea* weakly correlated with PC2 values.
- Decreases of the genus *Anaeroplasma* most strongly and those of *VaddinBB60* weakly correlated with PC2.

Conclusions

- TMEV infection changes relative abundance of four bacterial genera *Dorea*, *VadinBB60*, *Marvinbryantia*, and *Coprococcus*, which correlate with distinct gene expressions, including immunoglobulin genes.
- CNS TMEV infection increases the diversity of gut microbiota.
- Increased *S24-7* and decreased *Anaeroplasma* reflect CNS inflammation.

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