Herpes Simplex Virus Infection, Acyclovir and IVIG Treatment All Independently Cause Gut Dysbiosis.

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Abstract.

Herpes simplex virus 1 (HSV) is a ubiquitous human virus resident in a majority of the global population as a latent infection. Acyclovir (ACV), is the standard of care drug used to treat primary and recurrent infections, supplemented in some patients with intravenous immunoglobulin (IVIG) treatment to suppress deleterious inflammatory responses. We found that HSV, ACV and IVIG can all independently disrupt the gut bacterial community in a sex biased manner when given to uninfected mice. Treatment of HSV infected mice with ACV or IVIG alone or together revealed complex interactions between these drugs and infection that caused pronounced sex biased dysbiosis. ACV reduced *Bacteroidetes* levels in male but not female mice, while levels of the Anti-inflammatory Clostridia (AIC) were reduced in female but not male mice, which is significant as these taxa are associated with protection against the development of GVHD in hematopoietic stem cell transplant (HSCT) patients. Gut barrier dysfunction is associated with GVHD in HSCT patients and ACV also decreased *Akkermansia muciniphila,* which is important for maintaining gut barrier functionality. Cumulatively, our data suggest that long-term prophylactic ACV treatment of HSCT patients may contribute to GVHD and potentially impact immune reconstitution. These data have important implications for other clinical settings, including HSV eye disease and genital infections, where ACV is given long-term.

Author Summary.

Primary and reactivated HSV and VZV infections are treated with Acyclovir (ACV), an antiviral drug that blocks viral DNA synthesis. In some patients IVIG is used as adjunctive therapy to block deleterious inflammation. Long term preventative treatment of patients who receive stem transplants for various blood cancers has been successful in preventing life threatening reactivated HSV and VZV infections, but GVHD remains a major factor limiting transplant

success. Studies reported here reveal that HSV infection, ACV and IVIG given alone can all disrupt the gut microbiota and that complex interactions between these drugs and infection results in even more pronounced sex biased changes in the gut bacteria community structure. Importantly, ACV treatment decreased the levels of specific bacterial taxa, including the anti-inflammatory *Clostriodia* and *Bacteroidetes* that have been shown to protect against development of GVHD in stem cell transplant patients. These data suggest that long term preventative treatment of patients with ACV may contribute to GVHD in transplant patients and have negative consequences in other HSV induced diseases treated long term with ACV. The health effects of long term ACV and IVIG treatments warrant further clinical studies.

Introduction.

Herpes Simplex Virus type 1 (HSV), a ubiquitous human virus is the major cause of HSV encephalitis (HSE), the most prevalent sporadic encephalitis resulting from either primary infection or reactivation of latent virus. However, despite improved diagnostic procedures and effective antiviral therapies, most HSE survivors have persistent neurological impairments, including memory and behavior disturbances, dysphasia and seizures, and only 50-65% of these survivors return to independent living [1, 2]. A delay in initiating Acyclovir (ACV) treatment past the second hospital day is associated with poor neurological outcomes [3, 4]. Recent clinical trials evaluating prolonged oral ACV/valaciclovir (VACV) treatment following standard 14-day intravenous ACV treatment reported improved neurocognitive outcomes in neonates but not adults for reasons that are obscure [5, 6]. Although, it is generally accepted that replication induced pathology underlies HSV related neurological dysfunction, supporting experimental or clinical evidence is lacking. Overwhelming evidence has linked inflammation to the development of various neurological disorders and neuropsychiatric diseases, including Alzheimer's disease

(AD), schizophrenia, autism spectrum disorder (ASD), multiple sclerosis (MS), Parkinson's disease (PD), depression and anxiety [7-9].

Having unequivocally established that HSE arises from exaggerated CNS inflammatory responses and that the immunomodulatory activities of intravenous immunoglobulins (IVIG) can prevent HSE in a mouse model [10], we tested the hypothesis that persistent inflammation, which is documented in humans and mice after HSE [11-14], causes neurobehavioral impairments in survivors, that should be impeded by IVIG's anti-inflammatory activity [10]. Compared to treatment of HSV infected mice with ACV or PBS alone, treatment with ACV+IVIG from day 4 pi reduced CNS inflammation and anxiety, consistent with our hypothesis. Strikingly, development of learning and memory (LM) deficits that were evident only in female PBS treated mice, were inhibited by ACV treatment and counterintuitively, aggravated by ACV+IVIG treatment. Treatment of infected male mice with ACV+IVIG also impaired LM compared to ACV or PBS alone, revealing that IVIG antagonized the beneficial effects of ACV [15]. Intriguingly, the differential antagonistic effects of ACV+IVIG on cognitive behavior in HSV infected mice, compared to ACV and PBS treatment alone, were reflected in differential serum proteomic profiles [15]. These reported antagonistic effects of ACV and IVIG on LM present a conundrum, since they are at odds with the known mechanisms of action of these drugs.

Rapidly accumulating evidence is revealing the critical role of the microbiome in regulating brain homeostasis and function such that perturbation of the gut bacteria community structure and function is increasingly being implicated in a variety of neurodegenerative and neuropsychiatric diseases. In an effort to gain insight into how HSV induces LM impairment and the paradoxical effects of ACV and IVIG, we investigated a role for the gut microbiota. HSV infection, ACV and IVIG were all associated with significant disruption of the gut bacterial community structure that was sex biased. Furthermore, treating HSV infected mice with either

ACV or IVIG alone or both drugs together resulted in more pronounced sex-biased shifts in the gut bacterial community structure compared to uninfected mice. These results have significant clinical implications, particularly when patients receive prolonged ACV or IVIG treatment.

Results.

Equal numbers (n=8) of female and male C57BL/6 mice were bilaterally inoculated with virulent HSV1 strain 17+ ($1x10^5$ PFU/eye) by corneal scarification as previously described [15]. At day 4 post infection (pi), ACV was administered at 1.25 mg / mouse by intraperitoneal injection (ip) daily for 3 days, while IVIG was given as single dose of 25 mg/mouse by ip injection on day 4pi [15]. Fresh fecal pellets (n=1-2/ mouse) were collected on day 7 pi and stored at -80^{o}C until processed for Illumina 16S rRNA gene sequencing to determine the effects of infection and drug treatment on the gut microbiome. Normal male and female mice differed in gut bacteria composition and unexpectedly, HSV ocular infection caused further shifts in the gut bacteria community and amplified this sex difference, as shown in a PCoA plot of Hellinger beta diversity distance values for infected compared to uninfected male and female mice (**Figure 1A**; $P<0.05$, Adonis Tests). In addition, HSV infection had a greater effect on gut bacterial communities in males ($P=0.003$) compared to females ($P=0.011$) (**Figure 1A**). Significant differences were observed at the phyla level, particularly for firmicutes (**Figure 1B**) with more marked differences evident at the species level for *Clostridium aerotolerans* and other clostridial species, for example *Clostridium XIVa* that ferment carbohydrates in the gut resulting in production of short chain fatty acids (SFCs) that contribute to barrier integrity and also exhibit anti-inflammatory properties (**Figure 1C**). A notable difference was also observed for *Akkermansia muciniphila* that has many health promoting activities, including maintaining gut barrier health (**Figure 1C**).

Treating HSV infected mice with ACV from day 4 pi for three days resulted in even more drastic shifts in the gut bacteria composition and exaggerated sex differences (**Figure 2A**), than for infection alone. Considerable abundance changes were evident at the Phyla level for *Bacteroidetes*, *Firmicutes* and *Verrucomicrobia* (**Figure 2B**) and at the species level (**Figure 2C**). Notably, whereas HSV infection reduced the abundance of *Firmicutes* significantly in male but not female mice (**Figure 1B**), ACV reversed this effect restoring the abundance to the level in uninfected male mice, while also increasing the abundance in female mice (**Figure 2B** and **Figure 1B**). Notable abundance changes at the species level included drastic suppression of *Clostridium aerotolerans* in infected male mice compared to increased abundance in females (**Figure 1C**), while ACV treatment further increased this abundance only in females (**Figure 2C**). *Akkermansia muciniphila* abundance was increased by infection in male mice but reduced in females (**Figure 1C**), while ACV treatment resulted in total suppression of this species in female mice compared to a marked reduction in male mice (**Figure 2C**). There are many other similar changes in species abundance that are differentially impacted by ACV treatment in a sex-biased manner, indicative of complex interactions between infection, ACV effects on infected host cells, and bacteria, as well as metabolites produced by bacterial metabolism of ACV.

Treatment of uninfected mice with IVIG alone also shifted the gut bacteria community composition with a notable marked sex effect as determined by a beta diversity analysis (**Figure 3**). Males and females showed a major reduction in *A. muciniphila,* and a lesser reduction of *Verrucomicrobia* in males, compared to females that showed increased abundance of this phylum in response to IVIG treatment (**Figure 4**). The abundance of many other bacterial species was differentially altered by IVIG treatment of males and females, for example, *Clostridium aerotolerans*, *Bacteroides acidifaciens* and *Porphyromonadaceae* (**Figure 4B**). The response to IVIG was distinct in HSV infected mice, and the complex interactions between infection, ACV and IVIG were also evident at the phyla and species levels and were strongly sex biased as well

(**Figure 4A and 4B**). IVIG treatment decreased *A. muciniphila* abundance markedly in infected males and females as did ACV, whereas in contrast, treatment with ACV+IVIG caused a notable increase in its abundance, indicative of antagonistic effects of these two drugs in the context of infection (**Figure 4B**) In a similar vein, *C. aerotolerans* abundance increased markedly in males, but was unchanged in females treated with IVIG, while in contrast, it was strongly decreased in males but slightly increased in females treated with ACV alone. In contrast, treatment with ACV+IVIG suppressed an IVIG-induced increase in males and an ACV-induced increase in females, revealing antagonism between ACV and IVIG in the context of HSV infection (**Figure 4B**).

Patients with hematologic and other malignancies have benefited immensely from allogeneic hematopoietic stem cell transplantation (allo-HSCT or HSCT), which can be a potent curative immunotherapy. However, life threatening complications such as graft-versus-host disease (GVHD), relapse, and infections that include reactivated HSV and VZV limit its application [16]. HSV and varicella zoster (VZV) reactivation has been successfully suppressed by prophylactic ACV treatment, though ACV-resistant (ACVr) HSV is an emerging problem [17, 18]. Long term ACV prophylactic treatment is now routine for HSCT patients, because it was found to correlate with reduced HSV and ACVr HSV disease in those treated for longer than 1 year [19].

Given this routine clinical practice, we evaluated the effects of ACV on fecal bacteria, because gut microbes have been implicated in GVHD pathophysiology and because we posit that ACV contributes to the development of GVHD by changing the gut microbiota. First, we identified gut bacterial changes in humans with GVHD [20-30]. Next, we determined whether the ACV-induced changes that we detected in this mouse study matched those GVHD-associated changes. Whenever we identified taxa that were altered in both types of studies, the direction of the change was the same, and it was consistent with our hypothesis that ACV contributes to the

development of human GVHD by changing the gut microbiota. In the following, we describe these results, and we note that these ACV-induced changes were only observed in the HSV-infected mice and not in the uninfected mice.

Reduced levels of several taxa belonging to the phylum *Bacteroidetes* have been shown to be associated with GVHD, indicating that these gut bacteria may play a protective role. In a pediatric study, GVHD patients had lower levels of the family *Bacteroidaceae* and the genus *Parabacteroides* [30]. In a longitudinal study, pediatric patients that had lower levels of *Bacteroidetes* prior to HSCT were more likely to develop GVHD [24]. In our study, all three if these taxa were reduced by ACV treatment in male but not female mice (**Figure 5A**).

Reduced levels of Anti-Inflammatory Clostridia (AIC) have also been detected in human GVHD patients [20, 23-25, 27-30], indicating that these gut bacteria may play a protective role. This terminology was first introduced by Piper et al. [31] in the context of short bowel syndrome, and then introduced to the GVHD literature by Simms-Waldrip et al. [30]. AIC taxa include members of the families *Clostridiaceae*, *Erysipelotrichaceae*, *Eubacteriaceae*, *Lachnospiraceae* and *Ruminococcaceae*. In a pediatric study, decreases in *Blautia* and *Clostridium bolteae* were associated with the development of GVHD [30]. In an adult study, lower levels of *Blautia*, *Blautia hansenii*, and *Blautia stercoris* were associated with the development of GVHD [28]. In a longitudinal study, reduced levels of the *Blautia* before HSCT was shown to be a predictive marker for the development of GVHD [27]. In our study, all of these taxa were reduced by ACV treatment in female but not male mice (**Figure 5B**).

In a more detailed analysis of AIC bacteria, we observed that while HSV infection increased the abundance of *Blautia hansenii* only in males, ACV treatment reduced its abundance in females but had no effect on its abundance in males (**Supplemental Figure 1**). Remarkably,

a dramatic increase in *B. hansenii* in uninfected females was observed after IVIG treatment, and this increase was abrogated by ACV (compare NoHSV_F, NoHSV_IVIG_F and NoHSV_ACVplusIVIG_F) (**Supplemental Figure 1**), a result that supports sex-based differential effects of these drugs. However, during HSV infection, both IVIG and ACV reduced *B. hansenii* in females, whereas only IVIG reduced abundance in males. Interestingly, HSV infection significantly increased the abundance of the AIC genera *Blautia, Allobaculum,* and *Clostridium* XVIII but not *Turicibacter* in both males and females (**Supplemental Figure 2**). ACV treatment of HSV infected female mice resulted in significant decreases in the abundances of 4 AIC genera: *Blautia*, *Allobaculum*, *Clostridium* XVIII and *Turicibacter,* whereas in infected males, ACV decreased the abundance of *Marvinbryantia* and *Oscillibacter* (**Supplemental Figure 2**). In addition, ACV increased the abundance of *Turicibacter* in uninfected females but not males.

Finally, the two most abundant operational taxonomic units (OTUs), which exhibited a change in their relative abundances due to ACV treatment, were assigned to the family *Porphyromonadaceae* and the species *A. muciniphila* (**Figure 5C**). While we did not find these taxa associated with GVHD in prior human studies, GVHD has been associated with intestinal barrier dysfunction [32-36]. Supporting our hypothesis that ACV contributes to the development of GVHD by changing the gut microbiota, members of the *Porphyromonadaceae* have been shown to cause gut barrier dysfunction [37, 38], and our *Porphyromonadaceae* OTU was increased in its abundance by ACV. In addition, *A. muciniphila* was decreased by ACV treatment in our study, and it has been shown to strengthen gut barrier functioning [39-41].

Discussion.

Our intention in this brief report is to alert the scientific community and especially clinicians to the fact that HSV infection, the antiviral drug ACV, and the immunomodulatory biological, IVIG,

237 can all independently result in significant perturbations of the gut bacterial communities. Our data
238 reveal complex interactions between HSV infection and ACV or/and IVIG treatment that result in
239 marked alterations to gut bacterial communities. Although the clinical consequences of these
240 changes have not yet been elucidated, they could have profound implications in several settings
241 including HSCT-associated GVHD.
242
243 Though the mechanisms by which ocular HSV infection causes gut dysbiosis are unclear,
244 neuroinflammatory mechanisms and effects on the enteric nervous system via connected
245 brainstem neuronal circuits can be envisaged [15, 42]. Indeed, recent paradigm-shifting reports
246 reveal that peripheral neurons, including nociceptive and sensory neurons, can directly sense and
247 respond to environmental alarms by releasing neuropeptides that can regulate immune responses
248 in target organs including the gut [43, 44]. Persistence of gut dysbiosis was not evaluated here,
249 but results from a behavioral study alluded to earlier suggest long-term effects of infection and
250 drug treatment on gut bacterial ecology should be investigated [15]. Sex biased effects on HSV
251 induced dysbiosis merit further study, as these may involve microglial responses to HSV infection
252 and the microglial compartment is known to be regulated by the microbiota in a sex biased manner
253 [45-47].
254
255 The mechanism by which ACV, the standard antiviral for HSV infections, changes the gut
256 microbiota likely involves its uptake into bacteria. ACV is preferentially phosphorylated by the viral
257 encoded thymidine kinase (Tk) resulting in cell retention and eventual incorporation into viral DNA
258 resulting in inhibition of viral replication via DNA chain termination. Because Tk is conserved in
259 numerous bacterial species, ACV can be taken up and incorporated into DNA, resulting in
260 bactericidal effects [48-51]. Indeed, early studies on DNA replication mechanisms relied on
261 labeling bacterial DNA with tritiated thymidine and many bacterial taxa can be imaged using
262 nucleoside analogues such as 1-(2_-deoxy-2_-fluoro-_-D-arabinofuranosyl)-5-[125I] iodouracil

263 ([125I]FIAU) that are substrates for HSV Tk [52-55]. Incorporation of [*methyl*-^{3}H]thymidine into
264 DNA has been unequivocally demonstrated for members of the *Clostridium* genus [56] and our
265 data show ACV reduced the abundance of the *Blautia* genus (order *Clostridiales;* [57]) *Blautia*
266 *hansenii*, *Blautia stercoris*, and *Clostridium bolteae* in females but not males. Additionally,
267 interrogating the NCBI reference genome sequence for *Blautia hansenii* confirmed the presence
268 of a thymidine kinase enzyme. Our data are therefore consistent with ACV causing dysbiosis by,
269 at least in part, inhibiting the growth of various bacteria taxa via the Tk mechanism, though other
270 mechanisms involving bacterial metabolism of ACV cannot be excluded. Clearly, the mechanisms
271 by which ACV affects gut bacterial ecology are complex, which is further supported by the sex-
272 biased effects.
273
274 We also explored the effects of IVIG treatment alone and in combination with ACV in HSV-
275 infected and uninfected mice, because IVIG has been used to treat HSV encephalitis (HSE) and
276 is also a frontline therapy for autoimmune encephalitis, which is triggered by HSE and other insults
277 [58-60]. Moreover, IVIG is being evaluated in a randomized control trial for children with all-cause
278 encephalitis to determine whether neurological outcomes are improved compared to standard
279 antiviral therapy alone, which is similar to our behavioral study that generated paradoxical results
280 [15, 61]. Reports that IVIG's antigenic repertoire includes reactivities to a variety of gut commensal
281 antigens and metabolites have increased recently [62-64], which is consistent with a report that
282 gut commensals can somehow trigger systemic IgG responses under homeostatic conditions that
283 protect against systemic infection [65, 66]. We speculate that by neutralizing bacterial/host
284 antigens/metabolites, IVIG is able to influence host immunity, the nervous system, and other
285 physiological processes, resulting in perturbation of gut bacteria ecology. We speculate that the
286 disparate and complex effects of ACV and IVIG alone and in combination on the gut bacteria
287 ecology likely account for their antagonistic effects on cognitive behavior in mice latently infected
288 with HSV that we alluded to earlier [15].

289

290 This study has several limitations. Being exploratory in nature, analyses of the gut bacteria
291 were done at a single time point immediately after infection or drug treatment, rather than as a
292 longitudinal study that would have provided information on the persistence of the dysbiotic state
293 as well as mechanistic insights as to how HSV, ACV and IVIG provoke dysbiosis. Ideally, the
294 effects of ACV should be tested in latently infected mice, since virtually all HSCT patients harbor
295 latent HSV. However, because HSV infection alone disrupts the gut bacterial community,
296 assessing the effects of ACV on the gut bacteria community structure in the latently infected mice
297 would likely be difficult. Because ACV was given ip to mice but usually orally to HSCT patients
298 [67], its effects on the gut bacteria community maybe underestimated in our study.

299

300 Notwithstanding these caveats, our finding that ACV treatment of HSV infected mice
301 decreased the relative abundances of several bacterial taxa is important because these bacteria
302 have been negatively correlated with the induction of and mortality from GVHD in HSCT patients
303 [24, 27, 28, 30]. These results are also consistent with our hypothesis that ACV contributes to the
304 development of GVHD by changing the gut microbiota. In the context of allo-HSCT, GVHD occurs
305 when donor immune cells recognize recipient tissues as foreign, leading to immune-mediated
306 damage to several organs and tissues including the gastrointestinal tract. This has led
307 researchers to posit that the reduction of anti-inflammatory bacteria such as AIC contribute to
308 GVHD pathology [30]. The results from our study extend this hypothesis to include ACV treatment
309 as a putative contributor to GVHD, because ACV reduced AIC bacteria in the gut. ACV treatment
310 also decreased the relative abundances of several members of the *Bacteroidetes*, some of which
311 have been shown to exhibit anti-inflammatory properties [68-71]. More relevantly, the capsular
312 polysaccharide A (PSA) from *Bacteroides fragilis* reduced HSV-associated mortality in mice by
313 dramatically reducing immune-mediated inflammation [72]. In addition, the two most abundant
314 OTUs identified in our study, whose relative abundances were positively (*Porphyromonadaceae*)

and negatively (*A. muciniphila*) correlated with ACV treatment, have been shown to weaken [37, 38] and strengthen [39-41] gut barrier function, respectively. These results provide an additional link between ACV treatment and GVHD, because barrier dysfunction, which can cause systemic inflammation, is a hallmark of GVHD [32-36]. Finally, long-term ACV prophylaxis initiated early after HSCT might also impair immune reconstitution based on results from a study of antibiotic depletion of gut bacteria in a murine model of syngeneic bone marrow transplantation [73]. These tantalizing results warrant independent validation and further detailed studies using a murine autologous BMT model to more rigorously evaluate the impact of long-term ACV prophylaxis on GVHD and engraftment, because results from such studies might eventually lead to improved outcomes for HSCT patients. Ideally, such future studies should be performed with mice harboring wild microbiota, because several recent reports show that immune responses in mice with wild microbiomes model human immune responses more closely than conventional mice with SPF microbiota [74-76].

Materials and Methods.

Ethics Statement

All animal procedures were performed with prior approval of the City of Hope Institutional Animal Care and Use Committee (IACUC) under protocol # 07043 and within the framework of the Guide for the Care and Use of Laboratory Animals. C57BL6/J (B6) were bred in the vivarium at City of Hope.

Mouse Studies

Master stocks of HSV1 strain 17 composed of only of cell-released virus were prepared in and their titers determined on mycoplasma-free CV-1 cell monolayers. Single use aliquots of virus

in Hanks balanced salt solution supplemented with 2% fetal bovine serum were stored at -80°C. Male and female mice, 6–8 weeks of age, were infected with HSV1 17^{+}, a virulent strain. Mice were sedated with ketamine (60 mg/kg) and xylazine (5 mg/kg) prior to HSV inoculation by corneal scarification. B6 mice were bilaterally inoculated with 1x 10^{5} PFU per eye and monitored daily as previously described [15, 77].

Administration of Acyclovir and Intravenous Immunoglobulins.

ACV obtained from (APP Pharmaceuticals, Schaumburg, IL) was given at 50 mg/kg of body weight by intraperitoneal (ip) injection daily for 3 days starting on day 4 pi and PBS was given according to the same schedule to control mice. IVIG (Carimune, NF) obtained from CSL Behring (King of Prussia, PA, USA) was given ip as a single 0.5 ml dose (25 mg/mouse) on day 4 pi or it was given in combination with a 3 day course of ACV.

Illumina Bacterial 16S rRNA gene sequencing.

Illumina bacterial 16S rRNA gene libraries were constructed as follows. PCRs were performed in an MJ Research PTC-200 thermal cycler (Bio-Rad Inc., Hercules, CA, USA) as 25 µl reactions containing: 50 mM Tris (pH 8.3), 500 µg/ml bovine serum albumin (BSA), 2.5 mM $MgCl_2$, 250 µM of each deoxynucleotide triphosphate (dNTP), 400 nM of the forward PCR primer, 200 nM of each reverse PCR primer, 1 µl of DNA template, and 0.25 units JumpStart Taq DNA polymerase (Sigma-Aldrich, St. Louis, MO, USA). PCR primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) were used to targeted the 16S rRNA gene containing portions of the hypervariable regions V4 and V5, with the reverse primers including a 12-bp barcode [78]. Thermal cycling parameters were 94°C for 5 min; 35 cycles of 94°C for 20 s, 50°C for 20 s, and 72°C for 30 s, and followed by 72°C for 5 min. PCR

367 products were purified using the MinElute 96 UF PCR Purification Kit (Qiagen, Valencia, CA,
368 USA).
369
370 **16S rRNA gene data processing**.
371
372 We used the UPARSE pipeline for de-multiplexing, length trimming, quality filtering and
373 operational taxonomic units (OTU) picking using default parameters or recommended guidelines
374 that were initially described in [79] and which have been updated at
375 https://www.drive5.com/usearch/manual/uparse_pipeline.html. Briefly, after demultiplexing,
376 sequences were trimmed to a uniform length of 249 bp, then filtered at the recommended 1.0
377 expected error threshold. Sequences were then dereplicated and clustered into zero-radius OTUs
378 using the UNOISE3 algorithm [80], which also detects and removes chimeric sequences; this
379 method is based on making OTUs at 100% identity. An OTU table was then generated using the
380 otutab command. OTUs having non-bacterial DNA were identified by performing a local BLAST
381 search [81] of their seed sequences against the nt database. OTUs were removed if any of their
382 highest-scoring BLAST hits contained taxonomic IDs within Rodentia, Viridiplantae, Fungi, or
383 PhiX. Taxonomic assignments to the OTUs were performed with SINTAX [82] using RDP
384 Classifier 16S training set number 16 [83] as the reference database.
385
386 **16S rRNA gene data analyses**.
387 Beta diversity was measured using QIIME 1.9.1 [84] to calculate a Hellinger beta diversity
388 distance matrix, which was depicted using principle coordinates analysis (PCoA), and statistically
389 assessed by performing Adonis tests. Statistical differences among the taxa were determined
390 using edgeR [85, 86]. Taxa relative abundance figures were made using Prism (GraphPad, La
391 Jolla, CA). Comparative analyses of the bacterial taxa between human GVHD studies and our
392 mouse study excluded sequence-selective qPCR, because the selectivity of such assays is

393 questionable given the conserved nature of the 16S rRNA gene, and because the results of such
394 studies are not typically validated by sequence analyses. The bacterial sequences have been
395 deposited in the National Center for Biotechnology Information (NCBI)'s Sequence Read Archive
396 (SRA) under the BioProject Accession Number PRJNA549765.
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399 **References.**

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401 1. McGrath N, Anderson NE, Croxson MC, Powell KF. Herpes simplex encephalitis treated
402 with acyclovir: diagnosis and long term outcome. Journal of Neurology, Neurosurgery and
403 Psychiatry. 1997;63(3):321-6. doi: 10.1136/jnnp.63.3.321.
404 2. Raschilas F, Wolff M, Delatour Fdr, Chaffaut C, De Broucker T, Chevret S, et al. Outcome
405 of and Prognostic Factors for Herpes Simplex Encephalitis in Adult Patients: Results of a
406 Multicenter Study. Clin Infect Dis. 2002;35(3):254-60. doi: 10.1086/341405.
407 3. Kennedy PGE, Steiner I. Recent issues in herpes simplex encephalitis. Journal of
408 NeuroVirology. 2013;19(4):346-50. doi: 10.1007/s13365-013-0178-6.
409 4. Bradshaw MJ, Venkatesan A. Herpes Simplex Virus-1 Encephalitis in Adults:
410 Pathophysiology, Diagnosis, and Management. Neurotherapeutics. 2016;13(3):493-508. doi:
411 10.1007/s13311-016-0433-7.
412 5. Gnann JW, Sköldenberg B, Hart J, Aurelius E, Schliamser S, Studahl M, et al. Herpes
413 Simplex Encephalitis: Lack of Clinical Benefit of Long-term Valacyclovir Therapy. Clin Infect Dis.
414 2015;61(5):683-91. doi: 10.1093/cid/civ369.
415 6. Kimberlin DW, Whitley RJ, Wan W, Powell DA, Storch G, Ahmed A, et al. Oral Acyclovir
416 Suppression and Neurodevelopment after Neonatal Herpes. New England Journal of Medicine.
417 2011;365(14):1284-92. doi: doi:10.1056/NEJMoa1003509.
418 7. Chiara G, Marcocci M, Sgarbanti R, Civitelli L, Ripoli C, Piacentini R, et al. Infectious
419 Agents and Neurodegeneration. Molecular Neurobiology. 2012;46(3):614-38. doi:
420 10.1007/s12035-012-8320-7.
421 8. Hope S, Hoseth E, Dieset I, Mørch RH, Aas M, Aukrust P, et al. Inflammatory markers are
422 associated with general cognitive abilities in schizophrenia and bipolar disorder patients and
423 healthy controls. Schizophrenia Research. 2015;165(2-3):188-94. doi:
424 http://dx.doi.org/10.1016/j.schres.20151654.04.004.
425 9. Kuntz T, Gilbert J. Does the brain listen to the gut? eLife. 2016;5:e17052. doi:
426 10.7554/eLife.17052.
427 10. Ramakrishna C, Newo ANS, Shen Y-W, Cantin E. Passively Administered Pooled Human
428 Immunoglobulins Exert IL-10 Dependent Anti-Inflammatory Effects that Protect against Fatal HSV
429 Encephalitis. PLoS Pathog. 2011;7(6):e1002071. doi: 10.1371/journal.ppat.1002071.
430 11. Cantin EM, Hinton DR, Chen J, Openshaw H. Gamma interferon expression during acute
431 and latent nervous system infection by herpes simplex virus type 1. J Virol. 1995;69(8):4898-905.
432 12. Liu T, Tang Q, Hendricks RL. Inflammatory infiltration of the trigeminal ganglion after
433 herpes simplex virus type 1 corneal infection. J Virol. 1996;70(1):264-71.
434 13. Khanna KM, Lepisto AJ, Decman V, Hendricks RL. Immune control of herpes simplex
435 virus during latency. Current Opinion in Immunology. 2004;16(4):463-9.
436 14. Aurelius E, Forsgren M, Skoldenberg B, Strannegard O. Persistent intrathecal immune
437 activation in patients with herpes simplex encephalitis. J Infect Dis. 1993;168(5):1248-52.
438 15. Ramakrishna C, Golub MS, Chiang A, Hong T, Kalkum M, Cantin EM. Effects of Acyclovir
439 and IVIG on Behavioral Outcomes after HSV1 CNS Infection. Behavioural Neurology.
440 2017;2017:14. doi: 10.1155/2017/5238402.
441 16. Shono Y, van den Brink MRM. Gut microbiota injury in allogeneic haematopoietic stem
442 cell transplantation. Nature Reviews Cancer. 2018. doi: 10.1038/nrc.2018.10.
443 17. Frobert E, Burrel S, Ducastelle-Lepretre S, Billaud G, Ader F, Casalegno J-S, et al.
444 Resistance of herpes simplex viruses to acyclovir: An update from a ten-year survey in France.
445 Antiviral Research. 2014;111(0):36-41. doi: http://dx.doi.org/10.1016/j.antiviral.2014.08.013.
446 18. Baumrin E, Cheng MP, Kanjilal S, Ho VT, Issa NC, Baden LR. Severe Herpes Zoster
447 Requiring Intravenous Antiviral Treatment in Allogeneic Hematopoietic Cell Transplantation

448 Recipients on Standard Acyclovir Prophylaxis. Biology of Blood and Marrow Transplantation.
449 2019. doi: https://doi.org/10.1016/j.bbmt.2019.04.015.
450 19. Dadwal SS. Herpes Virus Infections Other than Cytomegalovirus in the Recipients of
451 Hematopoietic Stem Cell Transplantation. Infect Dis Clin North Am. 2019;33(2):467-84. doi:
452 https://doi.org/10.1016/j.idc.2019.02.012.
453 20. Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, et al. The effects of
454 intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell
455 transplantation. Blood. 2014;124(7):1174-82. Epub 2014/06/19. doi: 10.1182/blood-2014-02-
456 554725. PubMed PMID: 24939656; PubMed Central PMCID: PMCPMC4133489.
457 21. Weber D, Oefner PJ, Dettmer K, Hiergeist A, Koestler J, Gessner A, et al. Rifaximin
458 preserves intestinal microbiota balance in patients undergoing allogeneic stem cell
459 transplantation. Bone Marrow Transplant. 2016;51(8):1087-92. Epub 2016/03/22. doi:
460 10.1038/bmt.2016.66. PubMed PMID: 26999466.
461 22. Shono Y, Docampo MD, Peled JU, Perobelli SM, Velardi E, Tsai JJ, et al. Increased
462 GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem
463 cell transplantation in human patients and mice. Sci Transl Med. 2016;8(339):339ra71. Epub
464 2016/05/20. doi: 10.1126/scitranslmed.aaf2311. PubMed PMID: 27194729; PubMed Central
465 PMCID: PMCPMC4991773.
466 23. Peled JU, Devlin SM, Staffas A, Lumish M, Khanin R, Littmann ER, et al. Intestinal
467 Microbiota and Relapse After Hematopoietic-Cell Transplantation. J Clin Oncol.
468 2017;35(15):1650-9. Epub 2017/03/16. doi: 10.1200/jco.2016.70.3348. PubMed PMID:
469 28296584; PubMed Central PMCID: PMCPMC5455763.
470 24. Biagi E, Zama D, Nastasi C, Consolandi C, Fiori J, Rampelli S, et al. Gut microbiota
471 trajectory in pediatric patients undergoing hematopoietic SCT. Bone Marrow Transplant.
472 2015;50(7):992-8. doi: 10.1038/bmt.2015.16.
473 25. Jenq RR, Ubeda C, Taur Y, Menezes CC, Khanin R, Dudakov JA, et al. Regulation of
474 intestinal inflammation by microbiota following allogeneic bone marrow transplantation. J Exp
475 Med. 2012;209(5):903-11. Epub 2012/05/02. doi: 10.1084/jem.20112408. PubMed PMID:
476 22547653; PubMed Central PMCID: PMCPMC3348096.
477 26. Holler E, Butzhammer P, Schmid K, Hundsrucker C, Koestler J, Peter K, et al.
478 Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell
479 transplantation: loss of diversity is associated with use of systemic antibiotics and more
480 pronounced in gastrointestinal graft-versus-host disease. Biol Blood Marrow Transplant.
481 2014;20(5):640-5. Epub 2014/02/05. doi: 10.1016/j.bbmt.2014.01.030. PubMed PMID:
482 24492144; PubMed Central PMCID: PMCPMC4973578.
483 27. Biagi E, Zama D, Rampelli S, Turroni S, Brigidi P, Consolandi C, et al. Early gut microbiota
484 signature of aGvHD in children given allogeneic hematopoietic cell transplantation for
485 hematological disorders. BMC Med Genomics. 2019;12(1):49. Epub 2019/03/09. doi:
486 10.1186/s12920-019-0494-7. PubMed PMID: 30845942; PubMed Central PMCID:
487 PMCPMC6404274.
488 28. Jenq RR, Taur Y, Devlin SM, Ponce DM, Goldberg JD, Ahr KF, et al. Intestinal Blautia Is
489 Associated with Reduced Death from Graft-versus-Host Disease. Biol Blood Marrow Transplant.
490 2015;21(8):1373-83. Epub 2015/05/16. doi: 10.1016/j.bbmt.2015.04.016. PubMed PMID:
491 25977230; PubMed Central PMCID: PMCPMC4516127.
492 29. Weber D, Jenq RR, Peled JU, Taur Y, Hiergeist A, Koestler J, et al. Microbiota Disruption
493 Induced by Early Use of Broad-Spectrum Antibiotics Is an Independent Risk Factor of Outcome
494 after Allogeneic Stem Cell Transplantation. Biol Blood Marrow Transplant. 2017;23(5):845-52.
495 Epub 2017/02/25. doi: 10.1016/j.bbmt.2017.02.006. PubMed PMID: 28232086; PubMed Central
496 PMCID: PMCPMC5546237.
497 30. Simms-Waldrip TR, Sunkersett G, Coughlin LA, Savani MR, Arana C, Kim J, et al.
498 Antibiotic-Induced Depletion of Anti-inflammatory Clostridia Is Associated with the Development

499 of Graft-versus-Host Disease in Pediatric Stem Cell Transplantation Patients. Biol Blood Marrow
500 Transplant. 2017;23(5):820-9. Epub 2017/02/14. doi: 10.1016/j.bbmt.2017.02.004. PubMed
501 PMID: 28192251.
502 31. Piper HG, Fan D, Coughlin LA, Ho EX, McDaniel MM, Channabasappa N, et al. Severe
503 Gut Microbiota Dysbiosis Is Associated With Poor Growth in Patients With Short Bowel
504 Syndrome. JPEN J Parenter Enteral Nutr. 2017;41(7):1202-12. Epub 2016/07/14. doi:
505 10.1177/0148607116658762. PubMed PMID: 27406942.
506 32. Melson J, Jakate S, Fung H, Arai S, Keshavarzian A. Crypt loss is a marker of clinical
507 severity of acute gastrointestinal graft-versus-host disease. Am J Hematol. 2007;82(10):881-6.
508 Epub 2007/06/16. doi: 10.1002/ajh.20976. PubMed PMID: 17570511.
509 33. Spencer GD, Shulman HM, Myerson D, Thomas ED, McDonald GB. Diffuse intestinal
510 ulceration after marrow transplantation: a clinicopathologic study of 13 patients. Hum Pathol.
511 1986;17(6):621-33. Epub 1986/06/01. doi: 10.1016/s0046-8177(86)80135-6. PubMed PMID:
512 3011641.
513 34. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical
514 manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched
515 sibling donors. Transplantation. 1974;18(4):295-304. Epub 1974/10/01. doi: 10.1097/00007890-
516 197410000-00001. PubMed PMID: 4153799.
517 35. Ponec RJ, Hackman RC, McDonald GB. Endoscopic and histologic diagnosis of intestinal
518 graft-versus-host disease after marrow transplantation. Gastrointest Endosc. 1999;49(5):612-21.
519 Epub 1999/05/06. doi: 10.1016/s0016-5107(99)70390-1. PubMed PMID: 10228260.
520 36. Sale GE, Shulman HM, McDonald GB, Thomas ED. Gastrointestinal graft-versus-host
521 disease in man. A clinicopathologic study of the rectal biopsy. Am J Surg Pathol. 1979;3(4):291-
522 9. Epub 1979/08/01. PubMed PMID: 44107.
523 37. Nakajima M, Arimatsu K, Kato T, Matsuda Y, Minagawa T, Takahashi N, et al. Oral
524 Administration of P. gingivalis Induces Dysbiosis of Gut Microbiota and Impaired Barrier Function
525 Leading to Dissemination of Enterobacteria to the Liver. PLoS One. 2015;10(7):e0134234. Epub
526 2015/07/29. doi: 10.1371/journal.pone.0134234. PubMed PMID: 26218067; PubMed Central
527 PMCID: PMCPMC4517782.
528 38. Flak MB, Colas RA, Munoz-Atienza E, Curtis MA, Dalli J, Pitzalis C. Inflammatory arthritis
529 disrupts gut resolution mechanisms, promoting barrier breakdown by Porphyromonas gingivalis.
530 JCI Insight. 2019;4(13). Epub 2019/07/12. doi: 10.1172/jci.insight.125191. PubMed PMID:
531 31292292; PubMed Central PMCID: PMCPMC6629160.
532 39. van der Lugt B, van Beek AA, Aalvink S, Meijer B, Sovran B, Vermeij WP, et al.
533 Akkermansia muciniphila ameliorates the age-related decline in colonic mucus thickness and
534 attenuates immune activation in accelerated aging Ercc1 (-/Delta7) mice. Immun Ageing.
535 2019;16:6. Epub 2019/03/23. doi: 10.1186/s12979-019-0145-z. PubMed PMID: 30899315;
536 PubMed Central PMCID: PMCPMC6408808.
537 40. Grander C, Adolph TE, Wieser V, Lowe P, Wrzosek L, Gyongyosi B, et al. Recovery of
538 ethanol-induced Akkermansia muciniphila depletion ameliorates alcoholic liver disease. Gut.
539 2018;67(5):891-901. Epub 2017/05/28. doi: 10.1136/gutjnl-2016-313432. PubMed PMID:
540 28550049.
541 41. Wu W, Lv L, Shi D, Ye J, Fang D, Guo F, et al. Protective Effect of Akkermansia
542 muciniphila against Immune-Mediated Liver Injury in a Mouse Model. Front Microbiol.
543 2017;8:1804. Epub 2017/10/17. doi: 10.3389/fmicb.2017.01804. PubMed PMID: 29033903;
544 PubMed Central PMCID: PMCPMC5626943.
545 42. Gesser RM, Koo SC. Oral inoculation with herpes simplex virus type 1 infects enteric
546 neuron and mucosal nerve fibers within the gastrointestinal tract in mice. Journal of virology.
547 1996;70(6):4097-102. PubMed PMID: 8648749.

548 43. Basso L, Serhan N, Tauber M, Gaudenzio N. Peripheral neurons: Master regulators of
549 skin and mucosal immune response. European Journal of Immunology. 2019;0(0). doi:
550 10.1002/eji.201848027.
551 44. Pavlov VA, Tracey KJ. Neural regulation of immunity: molecular mechanisms and clinical
552 translation. Nat Neurosci. 2017;20(2):156-66. doi: 10.1038/nn.4477.
553 45. Lokensgard JR, Cheeran MC, Hu S, Gekker G, Peterson PK. Glial cell responses to
554 herpesvirus infections: role in defense and immunopathogenesis. J Infect Dis. 2002;186 Suppl
555 2:S171-9. doi: 10.1086/344272. PubMed PMID: 22311297.
556 46. Marques CP, Cheeran MCJ, Palmquist JM, Hu S, Urban SL, Lokensgard JR. Prolonged
557 Microglial Cell Activation and Lymphocyte Infiltration following Experimental Herpes Encephalitis.
558 J Immunol. 2008;181(9):6417-26.
559 47. Thion MS, Low D, Silvin A, Chen J, Grisel P, Schulte-Schrepping J, et al. Microbiome
560 Influences Prenatal and Adult Microglia in a Sex-Specific Manner. Cell. 2018;172:1-17. doi:
561 10.1016/j.cell.2017.11.042.
562 48. Black ME, Hruby DE. Nucleotide sequence of the Escherichia coli thymidine kinase gene
563 provides evidence for conservation of functional domains and quaternary structure. Molecular
564 Microbiology. 1991;5(2):373-9. doi: 10.1111/j.1365-2958.1991.tb02119.x.
565 49. Jeffrey WH, Paul JH. Thymidine uptake, thymidine incorporation, and thymidine kinase
566 activity in marine bacterium isolates. Applied and Environmental Microbiology. 1990;56(5):1367-
567 72.
568 50. Konrad A, Yarunova E, Tinta T, Piškur J, Liberles DA. The global distribution and evolution
569 of deoxyribonucleoside kinases in bacteria. Gene. 2012;492(1):117-20. doi:
570 https://doi.org/10.1016/j.gene.2011.10.039.
571 51. Lönnqvist B, Palmblad J, Ljungman P, Grimfors G, Järnmark M, Lerner R, et al. Oral
572 acyclovir as prophylaxis for bacterial infections during induction therapy for acute leukaemia in
573 adults. Support Care Cancer. 1993;1(3):139-44. doi: 10.1007/bf00366060.
574 52. Brewin N, Cairns J. State of the DNA replication fork during thymine deprivation of
575 Escherichia coli, as observed by pulse-labelling with [3H]thymidine. Journal of Molecular Biology.
576 1977;111(3):353-63. doi: https://doi.org/10.1016/S0022-2836(77)80057-0.
577 53. Bettegowda C, Foss CA, Cheong I, Wang Y, Diaz L, Agrawal N, et al. Imaging bacterial
578 infections with radiolabeled 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodouracil. Proceedings
579 of the National Academy of Sciences of the United States of America. 2005;102(4):1145-50. doi:
580 10.1073/pnas.0408861102.
581 54. Davis SL, Be NA, Lamichhane G, Nimmagadda S, Pomper MG, Bishai WR, et al. Bacterial
582 Thymidine Kinase as a Non-Invasive Imaging Reporter for <italic>Mycobacterium
583 tuberculosis</italic> in Live Animals. PLoS ONE. 2009;4(7):e6297. doi:
584 10.1371/journal.pone.0006297.
585 55. Peterson KL, Reid WC, Freeman AF, Holland SM, Pettigrew RI, Gharib AM, et al. The use
586 of 14C-FIAU to predict bacterial thymidine kinase presence: Implications for radiolabeled FIAU
587 bacterial imaging. Nuclear Medicine and Biology. 2013;40(5):638-42. doi:
588 http://dx.doi.org/10.1016/j.nucmedbio.2013.01.005.
589 56. Wellsbury P, Herbert RA, John Parkes R. Incorporation of [methyl-3H]thymidine by
590 obligate and facultative anaerobic bacteria when grown under defined culture conditions. FEMS
591 Microbiology Ecology. 1993;12(2):87-95. doi: 10.1111/j.1574-6941.1993.tb00020.x.
592 57. Jenq RR, Taur Y, Devlin SM, Ponce DM, Goldberg JD, Ahr KF, et al. Intestinal *Blautia* Is
593 Associated with Reduced Death from Graft-versus-Host Disease. Biology of Blood and Marrow
594 Transplantation. 2015;21(8):1373-83. doi: 10.1016/j.bbmt.2015.04.016.
595 58. Stingl C, Cardinale K, Van Mater H. An Update on the Treatment of Pediatric Autoimmune
596 Encephalitis. Current Treatment Options in Rheumatology. 2018;4(1):14-28. doi:
597 10.1007/s40674-018-0089-z.

59. Armangue T, Spatola M, Vlagea A, Mattozzi S, Cárceles-Cordon M, Martinez-Heras E, et al. Frequency, symptoms, risk factors, and outcomes of autoimmune encephalitis after herpes simplex encephalitis: a prospective observational study and retrospective analysis. The Lancet Neurology. 2018;17(9):760-72. doi: https://doi.org/10.1016/S1474-4422(18)30244-8.
60. Wekerle H. Brain Autoimmunity and Intestinal Microbiota: 100 Trillion Game Changers. Trends in Immunology. 2017;38(7):483-97. doi: https://doi.org/10.1016/j.it.2017.03.008.
61. Iro MA, Sadarangani M, Absoud M, Chong WK, Clark CA, Easton A, et al. /mmuno*g*lobuli*N* *i*n the *T*reatment of *E*ncephalitis (IgNiTE): protocol for a multicentre randomised controlled trial. BMJ Open. 2016;6(11). doi: 10.1136/bmjopen-2016-012356.
62. Ye SL, Lei M, Jiang P, Liu FJ, Wang ZK, Cao HJ, et al. Demonstration of the IgG antibody repertoire against the bacteria Escherichia coli in Chinese intravenous immunoglobulins. Journal of Pharmaceutical and Biomedical Analysis. 2017;133:8-14. doi: 10.1016/j.jpba.2016.10.018. PubMed PMID: WOS:000392894900002.
63. Uchimura Y, Fuhrer T, Li H, Lawson MA, Zimmermann M, Yilmaz B, et al. Antibodies Set Boundaries Limiting Microbial Metabolite Penetration and the Resultant Mammalian Host Response. Immunity. 2018;49(3):545-59.e5. doi: https://doi.org/10.1016/j.immuni.2018.08.004.
64. Schneider C, Smith DF, Cummings RD, Boligan KF, Hamilton RG, Bochner BS, et al. The human IgG anti-carbohydrate repertoire exhibits a universal architecture and contains specificity for microbial attachment sites. Science Translational Medicine. 2015;7(269):269ra1-ra1. doi: 10.1126/scitranslmed.3010524.
65. Zeng Melody Y, Cisalpino D, Varadarajan S, Hellman J, Warren HS, Cascalho M, et al. Gut Microbiota-Induced Immunoglobulin G Controls Systemic Infection by Symbiotic Bacteria and Pathogens. Immunity. 2016;44(1-12). doi: http://dx.doi.org/10.1016/j.immuni.2016.02.006.
66. Negm OH, MacKenzie B, Hamed MR, Ahmad OAJ, Shone CC, Humphreys DP, et al. Protective antibodies against Clostridium difficile are present in intravenous immunoglobulin and are retained in humans following its administration. Clinical & Experimental Immunology. 2017:n/a-n/a. doi: 10.1111/cei.12946.
67. Kakiuchi S, Tsuji M, Nishimura H, Yoshikawa T, Wang L, Takayama-Ito M, et al. Association of the Emergence of Acyclovir-Resistant Herpes Simplex Virus Type 1 With Prognosis in Hematopoietic Stem Cell Transplantation Patients. The Journal of Infectious Diseases. 2017;215(6):865-73. doi: 10.1093/infdis/jix042.
68. Shen Y, Giardino Torchia ML, Lawson GW, Karp CL, Ashwell JD, Mazmanian SK. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. Cell Host Microbe. 2012;12(4):509-20. Epub 2012/09/25. doi: 10.1016/j.chom.2012.08.004. PubMed PMID: 22999859; PubMed Central PMCID: PMCPMC3895402.
69. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A. 2010;107(27):12204-9. Epub 2010/06/23. doi: 10.1073/pnas.0909122107. PubMed PMID: 20566854; PubMed Central PMCID: PMCPMC2901479.
70. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008;453(7195):620-5. Epub 2008/05/30. doi: 10.1038/nature07008. PubMed PMID: 18509436.
71. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell. 2005;122(1):107-18. Epub 2005/07/13. doi: 10.1016/j.cell.2005.05.007. PubMed PMID: 16009137.
72. Ramakrishna C, Kujawski M, Chu H, Li L, Mazmanian SK, Cantin EM. Bacteroides fragilis polysaccharide A induces IL-10 secreting B and T cells that prevent viral encephalitis. Nat Commun. 2019;10(1):2153. Epub 2019/05/16. doi: 10.1038/s41467-019-09884-6. PubMed PMID: 31089128; PubMed Central PMCID: PMCPMC6517419.
73. Staffas A, Burgos da Silva M, Slingerland AE, Lazrak A, Bare CJ, Holman CD, et al. Nutritional Support from the Intestinal Microbiota Improves Hematopoietic Reconstitution after

649 Bone Marrow Transplantation in Mice. Cell Host & Microbe. 2018;23(4):447-57. doi:
650 10.1016/j.chom.2018.03.002.
651 74. Rosshart SP, Herz J, Vassallo BG, Hunter A, Wall MK, Badger JH, et al. Laboratory mice
652 born to wild mice have natural microbiota and model human immune responses. Science.
653 2019;365(6452):eaaw4361. doi: 10.1126/science.aaw4361.
654 75. Rosshart SP, Vassallo BG, Angeletti D, Hutchinson DS, Morgan AP, Takeda K, et al. Wild
655 Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. Cell.
656 2017;171(5):1015-28.e13
657 . doi: 10.1016/j.cell.2017.09.016.
658 76. Viney M, Riley EM. The Immunology of Wild Rodents: Current Status and Future
659 Prospects. Frontiers in Immunology. 2017;8(1481). doi: 10.3389/fimmu.2017.01481.
660 77. Lundberg P, Ramakrishna C, Brown J, Tyszka JM, Hamamura M, Hinton DR, et al. The
661 immune response to herpes simplex virus type 1 infection in susceptible mice is a major cause of
662 central nervous system pathology resulting in fatal encephalitis. J Virol. 2008;82(14):7078-88. doi:
663 10.1128/JVI.00619-08. PubMed PMID: 18480436; PubMed Central PMCID: PMCPMC2446972.
664 78. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et
665 al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc
666 Natl Acad Sci U S A. 2011;108. doi: 10.1073/pnas.1000080107.
667 79. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat
668 Methods. 2013;10. doi: 10.1038/nmeth.2604.
669 80. Edgar RC. UNOISE2: Improved error-correction for Illumina 16S and ITS amplicon reads.
670 bioRxiv. 2016. doi: http://dx.doi.org/10.1101/081257doi:http://dx.doi.org/10.1101/081257.
671 81. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool.
672 J Mol Biol. 1990;215(3):403-10. Epub 1990/10/05. doi: 10.1016/s0022-2836(05)80360-2.
673 PubMed PMID: 2231712.
674 82. Edgar RC. Edgar RC. 2016. SINTAX, a simple non-Bayesian taxonomy classifier for 16S
675 and ITS sequences. bioRxiv. 2016. doi:
676 :http://dx.doi.org/10.1101/074161doi:http://dx.doi.org/10.1101/074161.
677 83. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal Database
678 Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 2014;42(Database
679 issue):D633-42. Epub 2013/11/30. doi: 10.1093/nar/gkt1244. PubMed PMID: 24288368; PubMed
680 Central PMCID: PMCPMC3965039.
681 84. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al.
682 QIIME allows analysis of high-throughput community sequencing data. Nature methods.
683 2010;7(5):335-6.
684 85. McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq
685 experiments with respect to biological variation. Nucleic Acids Res. 2012;40(10):4288-97. Epub
686 2012/01/31. doi: 10.1093/nar/gks042. PubMed PMID: 22287627; PubMed Central PMCID:
687 PMCPMC3378882.
688 86. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential
689 expression analysis of digital gene expression data. Bioinformatics. 2010;26(1):139-40. doi:
690 10.1093/bioinformatics/btp616.
691