GABA administration limits viral replication and pneumonitis in a mouse model of COVID-19

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Abstract

Despite the availability of vaccines for COVID-19, serious illness and death induced by coronavirus infection will remain a global health burden because of vaccination hesitancy, possible virus mutations, and the appearance of novel coronaviruses. Accordingly, there is a need for new approaches to limit severe illness stemming from coronavirus infections. Cells of the immune system and lung epithelia express receptors for GABA (GABA-Rs), a widely used neurotransmitter within the CNS. GABA-R agonists have anti-inflammatory effects and can limit acute lung injury. We previously showed that GABA treatment effectively reduced disease severity and death rates in mice following infection with a coronavirus (MHV-1) which provides a potentially lethal model of COVID-19. Here, we report that GABA treatment also reduced viral load in the lungs, suggesting that GABA-Rs may provide a new druggable target to limit pulmonary coronavirus replication. Histopathological analysis revealed that GABA treatment reduced lung inflammatory infiltrates and damages. Since GABA is safe for human consumption, inexpensive, and available worldwide, it is a promising candidate to help treat COVID-19.

Introduction

While GABA-Rs are well known for their role in neurotransmission in the CNS, these receptors are also found on some cells in the periphery, most notably for our studies, on some cells of the immune system and lung epithelia. The biological roles of GABA-Rs on immune cells are not yet well understood, but there is a growing body of evidence that the activation of these receptors has immunoregulatory actions. Rodent and human macrophages and dendric cells express both GABA-Rs and GABA and GABA-R agonists inhibit their inflammatory activity (1-6). T cells also express GABA-Rs (4-8). The administration of GABA-R agonists inhibits autoreactive Th1 and Th17 cells while promoting $CD4^+$ and $CD8^+$ Treg responses (5, 6, 9) and ameliorates autoimmune disease in mouse models of type 1 diabetes (T1D), multiple sclerosis, and rheumatoid arthritis, and also limits inflammation in murine type 2 diabetes (1, 5, 6, 10, 11). Human immune cells also express GABA-Rs and GABA inhibits secretion of IL-6, TNF, IL-17A, CXCL10/IP-10, CCL4, CCL20, and MCP-3 from anti-CD3 stimulated PBMC from T1D patients (7). The ability of GABA-R agonists to inhibit the production of number of inflammatory factors is of potential interest for helping to treat COVID-19 since high levels of some of these inflammatory factors in patient sera is associated with the development of severe COVID-19 (12-15).

Lung epithelial cells also express GABA-Rs, specifically the $GABA_A$-R subtype. GABA and $GABA_A$-R positive allosteric modulators (PAMs) have been shown to reduce inflammation and improve alveolar fluid clearance and lung functional recovery in different rodent models of acute lung injury (16-23), as well as limit pulmonary inflammatory responses and improve clinical outcomes in ventilated human patients (24-26). $GABA_A$-R PAMs reduce macrophage infiltrates and inflammatory cytokine levels in bronchoalveolar lavage fluid (BALF) and limit inflammatory responses by rodent and human macrophages (3, 22, 27-31). GABA application reduces the secretion of inflammatory factors from LPS-stimulated human bronchial epithelial cells *in vitro* (17). Finally, GABA can inhibit platelet aggregation (32), which is important because pulmonary thrombosis often occurs in critically ill COVID-19 patients (33, 34).

Mouse hepatitis virus (MHV)-1 is a pneumotropic beta-coronavirus that is widely used as a safe model of SARS-CoV and SARS-CoV-2 infection (35-38). Intranasal inoculation with $\geq 5 \times 10^3$ plaque-forming units (PFU) of MHV-1 in A/J mice induces acute pneumonitis and acute respiratory distress syndrome with a high lethality rate. The MHV-1-infected mice develop clinical symptoms and pathological features similar to those in severely ill COVID-19 patients, including high levels of pulmonary edema, pneumonitis, dense macrophage infiltrates, hyaline membranes, fibrin deposits, accompanied by loss of body weight and respiratory distress (35-38). We previously showed that GABA treatment just after MHV-1 inoculation, or after the appearance of disease symptoms, very effectively protected the mice from severe illness and death (39). Here, we report that GABA treatment also reduced viral loads and pathological findings in their lungs. We discuss possible mechanisms underlying these observations.

Materials and methods

Mice. Female A/J mice (8 weeks in age) were purchased from the Jackson Laboratory and maintained in microisolator cages and fed with a standard diet and water *ad libitum*. This study was carried out in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocols for all experiments using vertebrate animals were approved by the Animal Research Committee at UCLA (approval # ARC-2020-122) and were carried out in compliance with the ARRIVE guidelines.

Reagents. GABA (stock #A2129) was purchased from Millipore-Sigma (St. Louis, MO, USA).

Virus. MHV-1, DBT cells, and HeLa-CECAM1 were generously provided by Dr. Stanley Perlman (University of Iowa). MHV-I virus was prepared and titered as previously described (35-39).

Viral infection and GABA treatment. At 9 weeks in age, individual A/J mice were anesthetized and inoculated intranasally with 5 x 10^3 PFU MHV-1 in 50 μl cold Dulbecco's modified Eagle's medium (DMEM). The mice were immediately randomized and provided with plain water (controls) or water that contained GABA (20 mg/mL) as per (9, 39) for the entirety of the observation period. The mice treated with GABA on day 0 are referred to as the $GABA_0$ group. Some virus-inoculated mice were provided with plain water for 2 days and treated with GABA beginning on day 3 post-inoculation and are referred to as the $GABA_3$ group. The mice were euthanized at 3 or 6 days post-infection and their right and left lungs were dissected for measurements of viral loads and histology, respectively.

Viral titers. Frozen lung samples were dounce-homogenized into 1 mL of ice-cold DMEM with 10% fetal calf serum and homogenized with 1 mm glass beads using a Qiagen TissueLyser-LT at 50 Hz for 6x1 min. The viral titers in the supernatants were determined by endpoint dilution (40) in HeLa-CEACAM1 cells (85% confluent, 5 x 10^4

cells/well) using the Spearman-Kärber formula (41) to calculate 50% tissue culture infectious dose ($TCID_{50}$).

Hematoxylin and eosin staining of lung sections. Their left lungs were fixed in 10% neutral buffered formalin and embedded in paraffin. Lung tissue sections (5 μm) were routine-stained with hematoxylin and eosin. Five images from each mouse were captured under a light microscope at 200 x magnification. The degrees of pathological changes were scored, based on the number of hyaline membranes, % of pulmonary areas with obvious inflammatory infiltrates in lung parenchyma, and the % of area with inflammatory consolidation within the total area of the section. The total numbers of hyaline-like membranes with, or without, cell debris or hyaline-like deposition in alveoli of the lung tissue section were scored as 0: none detectable; 1: 1-5; 2: 5-10; 3: >10. The areas of lung inflammation and hemorrhage in one lung section were estimated and the severity of inflammation and hemorrhage in the section was scored as 1: mild, 2: moderate; 3, marked, 4: severe. Accordingly, an inflammatory score in each mouse was obtained by % of lung areas x severity score. The areas of lung consolidation were estimated in the lung section and scored as 1: <10%; 2:11-25%: 3:26-50%; 4:>50%. Finally, the pneumonitis score of individual mice = (% of inflammation areas x severity score) + lung consolidation score + hyaline membrane score with a maximum score of 11.

Results

A/J mice were inoculated intranasally with MHV-1 (5 x 10^3 PFU) and then randomized to receive plain water (controls) or water containing GABA (20 mg/mL, as in (39)) for the remainder of the study. Mice from these groups were euthanized 3 or 6 days post-infection and the virial load in their lungs was determined. Concurrently, another group of MHV-1 inoculated mice was given water containing GABA beginning at 3 days post-infection and the viral load in their lungs was determined 6 days post-infection.

At three days post-infection the mean viral load in the lungs of mice given plain water was about 7-fold higher than that in mice given GABA immediately after infection ($p<0.5$, Fig. 1). Thereafter, the viral load in the lungs declined as expected, and by day 6 post-infection the viral load in the lungs of control mice were about twice that in the lungs of mice given GABA either immediately or 3 days post-infection, although these differences were not statistically significant (Fig. 1). Thus, early GABA treatment reduced viral loads in the lungs of mice.

Histological evaluation of lung sections from mice given GABA immediately following MHV-1 inoculation revealed reduced inflammatory infiltrates, hyaline-like membrane formation and fibrin deposits in the alveoli at 3 days post-infection, relative to that in control MHV-1 inoculated mice (Fig. 2A). Thus, GABA treatment limited the MHV-1 induced lung damage in A/J mice.

Discussion

Our previous study showed that GABA treatment beginning just after MHV-1 inoculation or after the appearance of symptoms rapidly curtailed disease progression (39). GABA-treated mice also had smaller lung coefficient indexes (indicative of less inflammation and edema). Thus, GABA-R activation can limit a very acute and highly lethal viral infection-induced pulmonary inflammation, a property that heretofore was unknown. Here, we show that early GABA treatment reduced MHV-1 replication in the lungs of mice when measured near the time of peak viral load in this model (35-37), which may have contributed to the better outcomes observed in GABA-treated animals. Hence, GABA-R agonists may provide a new approach to limit viral replication in the lungs.

We can envision a number of ways that GABA may have limited viral replication, including: 1) The lung alveolar cells of rodents and humans express $GABA_A$-Rs (30, 42). While the activation of $GABA_A$-R's Cl^- channels on neurons leads to Cl- influx and hyperpolarization, the activation of $GABA_A$-Rs on ATII cells induces Cl^- efflux and greater membrane depolarization (30, 42). Because coronaviruses promote Ca^{2+} influx to enhance their replication (43, 44), the activation of ATII $GABA_A$-Rs and the ensuing Cl^- efflux and membrane depolarization may limit the influx of extracellular Ca^{2+}, making the cellular environment less conducive to viral replication. 2) Activation of $GABA_A$-Rs on lung alveolar and large airway epithelial cells may have A) altered the secretion of immune signaling molecules from infected cells, B) altered alveolar surfactant production/absorption, and/or C) altered inflammatory responses and autophagy (45) and, D) reduced the expression of the MHV-1 receptor CAECAM1 in ways that limited virus production. Further detailed studies are needed to evaluate whether these factors, and/or others, contributed to the observed reduction in viral load.

Histopathological analysis of lungs from mice that received GABA just after MHV-1 inoculation revealed that three days post-infection these mice had significantly reduced lung inflammation and markers of lung damage relative to that in control mice. There are a number of different biological processes through which GABA treatment may have ameliorated the severity of pneumonitis: 1) GABA can inhibit macrophage and dendritic cell inflammatory activities (1-3, 29, 46, 47). Likewise, $GABA_A$-R PAMs

reduce the numbers of macrophages in broncholavage fluid, lung secretion of inflammatory cytokines, and inflammatory responses by rodent and human macrophages (22, 27-31). $GABA_A$-R agonists also inhibit activated Th17 and Th1 responses and promote $CD4^+$ and $CD8^+$ Tregs, however, since adaptive immune responses take time to arise, these abilities are unlikely to have contributed to GABA's ability to attenuate disease soon after MHV-1 infection. These effects on adaptive immune responses may be relevant for treating COVID-19 which has a longer disease course and in which high levels of circulating Th1, Th17, and Th2-secreted proteins are associated with severe illness (12, 13). 2) By reducing viral loads in the lungs, GABA treatment may have limited dysregulated inflammatory responses to the infection. 3) GABA and $GABA_A$-R PAMs reduce inflammation and improve alveolar fluid clearance and lung functional recovery in animal models of acute lung injury (16-18, 20-23) and in ventilated patients (24-26) and could have exerted similar actions in the MHV-1 infected mice. 4) GABA and $GABA_A$-R agonist treatments increase macrophage autophagy (45). In murine models of pneumatic bacterial infections, $GABA_A$-R agonists reduced bacterial load and TNFα and IL-6 levels in the lungs and protected the mice against illness (45). 5) GABA inhibits platelet aggregation (32)—this may be an important property because pulmonary thrombosis is increased in critically ill COVID-19 patients (33, 34). Thus, treatment with GABA may have led to better outcomes in MHV-1 infected mice through multiple and diverse pathways.

Much remains to be learned about the mechanisms by which GABA-R activation protected MHV-1 infected mice from severe pneumonitis and whether these observations extend to SARS-CoV-2 infections. Given that GABA can affect many biological processes and that viral infection is a very dynamic process it is clear that GABA-R agonist dosing needs to be carefully studied and optimized for different stages of coronavirus infection. Our observations provide a spring board for future investigations into whether the GABA system can be modulated to limit severe illness due to SARS-CoV-2, future novel coronavirus outbreaks, and other respiratory disorders.

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Disclosures. DLK and JT are inventors of GABA-related patents. DLK serves on the Scientific Advisory Board of Diamyd Medical. BM has no financial conflicts of interest.

Author Contributions. Conceived and designed the experiments: JT, DLK; Performed the experiments: JT, BM; Analyzed the data: JT, DLK. Wrote the paper: JT, DLK. Drs. Daniel Kaufman and Jide Tian are guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final manuscript as submitted.

References

1. Tian J, Yong J, Dang H, Kaufman DL. Oral GABA treatment downregulates inflammatory responses in a mouse model of rheumatoid arthritis. Autoimmunity. 2011;44:465-70. PubMed PMID: 21604972.
2. Bhat R, Axtell R, Mitra A, Miranda M, Lock C, Tsien RW, Steinman L. Inhibitory role for GABA in autoimmune inflammation. Proc Natl Acad Sci U S A. 2010;107(6):2580-5. PubMed PMID: 20133656; PMCID: PMC2823917.
3. Januzi L, Poirier JW, Maksoud MJE, Xiang YY, Veldhuizen RAW, Gill SE, Cregan SP, Zhang H, Dekaban GA, Lu WY. Autocrine GABA signaling distinctively regulates phenotypic activation of mouse pulmonary macrophages. Cell Immunol. 2018;332:7-23. Epub 2018/07/19. doi: 10.1016/j.cellimm.2018.07.001. PubMed PMID: 30017085.
4. Prud'homme GJ, Glinka Y, Hasilo C, Paraskevas S, Li X, Wang Q. GABA protects human islet cells against the deleterious effects of immunosuppressive drugs and exerts immunoinhibitory effects alone. Transplantation. 2013;96(7):616-23. doi: 10.1097/TP.0b013e31829c24be. PubMed PMID: 23851932.
5. Tian J, Dang H, O'Laco K, Song M, Tiu B-C, S G, Zakarian C, Kaufman D. Homotaurine treatment enhances CD4+ and CD8+ Treg responses and synergizes with low-dose anti-CD3 to enhance diabetes remission in type 1 diabetic mice. ImmuoHorizons. 2019:Oct 21;3(10):498-510. doi: 10.4049/immunohorizons.1900019; PMCID: PMC6823932
6. Tian J, Dang H, Wallner M, Olsen R, Kaufman DL. Homotaurine, a safe blood-brain barrier permeable GABAA-R-specific agonist, ameliorates disease in mouse models of multiple sclerosis. Sci Rep. 2018;8(1):16555. Epub 2018/11/10. doi: 10.1038/s41598-018-32733-3. PubMed PMID: 30410049; PMCID: PMC6224391.
7. Bhandage AK, Jin Z, Korol SV, Shen Q, Pei Y, Deng Q, Espes D, Carlsson PO, Kamali-Moghaddam M, Birnir B. GABA Regulates Release of Inflammatory Cytokines From Peripheral Blood Mononuclear Cells and CD4(+) T Cells and Is Immunosuppressive in Type 1 Diabetes. EBioMedicine. 2018;30:283-94. Epub

2018/04/09. doi: 10.1016/j.ebiom.2018.03.019. PubMed PMID: 29627388; PMCID: PMC5952354.

8. Tian J, Chau C, Hales TG, Kaufman DL. GABA(A) receptors mediate inhibition of T cell responses. J Neuroimmunol. 1999;96(1):21-8.

9. Tian J, Dang H, Nguyen AV, Chen Z, Kaufman DL. Combined therapy with GABA and proinsulin/alum acts synergistically to restore long-term normoglycemia by modulating T-cell autoimmunity and promoting beta-cell replication in newly diabetic NOD mice. Diabetes. 2014;63(9):3128-34. Epub 2014/08/26. doi: 10.2337/db13-1385. PubMed PMID: 25146474; PMCID: PMC4141368.

10. Tian J, Lu Y, Zhang H, Chau CH, Dang HN, Kaufman DL. Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model. J Immunol. 2004;173(8):5298-304. PubMed PMID: 15470076.

11. Tian J, Dang HN, Yong J, Chui WS, Dizon MP, Yaw CK, Kaufman DL. Oral treatment with gamma-aminobutyric acid improves glucose tolerance and insulin sensitivity by inhibiting inflammation in high fat diet-fed mice. PLoS One. 2011;6(9):e25338. doi: 10.1371/journal.pone.0025338. PubMed PMID: 21966503; PMCID: PMC3178643.

12. Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, Levantovsky R, Malle L, Moreira A, Park MD, Pia L, Risson E, Saffern M, Salome B, Esai Selvan M, Spindler MP, Tan J, van der Heide V, Gregory JK, Alexandropoulos K, Bhardwaj N, Brown BD, Greenbaum B, Gumus ZH, Homann D, Horowitz A, Kamphorst AO, Curotto de Lafaille MA, Mehandru S, Merad M, Samstein RM, Sinai Immunology Review P. Immunology of COVID-19: Current State of the Science. Immunity. 2020. Epub 2020/06/09. doi: 10.1016/j.immuni.2020.05.002. PubMed PMID: 32505227; PMCID: PMC7200337

13. Lucas C, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, Ellingson MK, Mao T, Oh JE, Israelow B, Takahashi T, Tokuyama M, Lu P, Venkataraman A, Park A, Mohanty S, Wang H, Wyllie AL, Vogels CBF, Earnest R, Lapidus S, Ott IM, Moore AJ, Muenker MC, Fournier JB, Campbell M, Odio CD, Casanovas-Massana A, Yale IT, Herbst R, Shaw AC, Medzhitov R, Schulz WL, Grubaugh ND, Dela Cruz C, Farhadian S, Ko AI, Omer SB, Iwasaki A. Longitudinal analyses reveal immunological misfiring in

severe COVID-19. Nature. 2020;584(7821):463-9. Epub 2020/07/28. doi: 10.1038/s41586-020-2588-y. PubMed PMID: 32717743.

14. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Semin Immunopathol. 2017;39(5):529-39. Epub 2017/05/04. doi: 10.1007/s00281-017-0629-x. PubMed PMID: 28466096; PMCID: PMC7079893.

15. Sariol A, Perlman S. Lessons for COVID-19 Immunity from Other Coronavirus Infections. Immunity. 2020;53(2):248-63. Epub 2020/07/28. doi: 10.1016/j.immuni.2020.07.005. PubMed PMID: 32717182; PMCID: PMC7359787.

16. Huang T, Zhang Y, Wang C, Gao J. Propofol reduces acute lung injury by up-regulating gamma-aminobutyric acid type a receptors. Exp Mol Pathol. 2019;110:104295. Epub 2019/08/17. doi: 10.1016/j.yexmp.2019.104295. PubMed PMID: 31419406.

17. Fortis S, Spieth PM, Lu WY, Parotto M, Haitsma JJ, Slutsky AS, Zhong N, Mazer CD, Zhang H. Effects of anesthetic regimes on inflammatory responses in a rat model of acute lung injury. Intensive Care Med. 2012;38(9):1548-55. Epub 2012/06/20. doi: 10.1007/s00134-012-2610-4. PubMed PMID: 22711173; PMCID: PMC4896809.

18. Chintagari NR, Liu L. GABA receptor ameliorates ventilator-induced lung injury in rats by improving alveolar fluid clearance. Crit Care. 2012;16(2):R55. Epub 2012/04/07. doi: 10.1186/cc11298. PubMed PMID: 22480160; PMCID: PMC3681384.

19. Jin S, Merchant ML, Ritzenthaler JD, McLeish KR, Lederer ED, Torres-Gonzalez E, Fraig M, Barati MT, Lentsch AB, Roman J, Klein JB, Rane MJ. Baclofen, a GABABR agonist, ameliorates immune-complex mediated acute lung injury by modulating pro-inflammatory mediators. PLoS One. 2015;10(4):e0121637. doi: 10.1371/journal.pone.0121637. PubMed PMID: 25848767; PMCID: PMC4388838.

20. Voigtsberger S, Lachmann RA, Leutert AC, Schlapfer M, Booy C, Reyes L, Urner M, Schild J, Schimmer RC, Beck-Schimmer B. Sevoflurane ameliorates gas exchange and attenuates lung damage in experimental lipopolysaccharide-induced lung injury. Anesthesiology. 2009;111(6):1238-48. Epub 2009/11/26. doi: 10.1097/ALN.0b013e3181bdf857. PubMed PMID: 19934867.

21. Faller S, Strosing KM, Ryter SW, Buerkle H, Loop T, Schmidt R, Hoetzel A. The volatile anesthetic isoflurane prevents ventilator-induced lung injury via phosphoinositide 3-kinase/Akt signaling in mice. Anesth Analg. 2012;114(4):747-56. Epub 2012/03/03. doi: 10.1213/ANE.0b013e31824762f0. PubMed PMID: 22383671.
22. Taniguchi T, Yamamoto K, Ohmoto N, Ohta K, Kobayashi T. Effects of propofol on hemodynamic and inflammatory responses to endotoxemia in rats. Crit Care Med. 2000;28(4):1101-6. Epub 2000/05/16. doi: 10.1097/00003246-200004000-00032. PubMed PMID: 10809290.
23. Lin X, Ju YN, Gao W, Li DM, Guo CC. Desflurane Attenuates Ventilator-Induced Lung Injury in Rats with Acute Respiratory Distress Syndrome. Biomed Res Int. 2018;2018:7507314. Epub 2018/04/20. doi: 10.1155/2018/7507314. PubMed PMID: 29670906; PMCID: PMC5833253.
24. Mahmoud K, Ammar A. Immunomodulatory Effects of Anesthetics during Thoracic Surgery. Anesthesiol Res Pract. 2011;2011:317410. Epub 2011/11/24. doi: 10.1155/2011/317410. PubMed PMID: 22110498; PMCID: PMC3205595.
25. De Conno E, Steurer MP, Wittlinger M, Zalunardo MP, Weder W, Schneiter D, Schimmer RC, Klaghofer R, Neff TA, Schmid ER, Spahn DR, Z'Graggen B R, Urner M, Beck-Schimmer B. Anesthetic-induced improvement of the inflammatory response to one-lung ventilation. Anesthesiology. 2009;110(6):1316-26. Epub 2009/05/07. doi: 10.1097/ALN.0b013e3181a10731. PubMed PMID: 19417610.
26. Schilling T, Kozian A, Kretzschmar M, Huth C, Welte T, Buhling F, Hedenstierna G, Hachenberg T. Effects of propofol and desflurane anaesthesia on the alveolar inflammatory response to one-lung ventilation. Br J Anaesth. 2007;99(3):368-75. Epub 2007/07/11. doi: 10.1093/bja/aem184. PubMed PMID: 17621602.
27. Kochiyama T, Li X, Nakayama H, Kage M, Yamane Y, Takamori K, Iwabuchi K, Inada E. Effect of Propofol on the Production of Inflammatory Cytokines by Human Polarized Macrophages. Mediators Inflamm. 2019;2019:1919538. Epub 2019/04/23. doi: 10.1155/2019/1919538. PubMed PMID: 31007601; PMCID: PMC6441544.
28. Forkuo GS, Nieman AN, Kodali R, Zahn NM, Li G, Rashid Roni MS, Stephen MR, Harris TW, Jahan R, Guthrie ML, Yu OB, Fisher JL, Yocum GT, Emala CW, Steeber DA, Stafford DC, Cook JM, Arnold LA. A Novel Orally Available Asthma Drug

Candidate That Reduces Smooth Muscle Constriction and Inflammation by Targeting GABAA Receptors in the Lung. Mol Pharm. 2018;15(5):1766-77. Epub 2018/03/27. doi: 10.1021/acs.molpharmaceut.7b01013. PubMed PMID: 29578347; PMCID: PMC5954213.

29. Wheeler DW, Thompson AJ, Corletto F, Reckless J, Loke JC, Lapaque N, Grant AJ, Mastroeni P, Grainger DJ, Padgett CL, O'Brien JA, Miller NG, Trowsdale J, Lummis SC, Menon DK, Beech JS. Anaesthetic impairment of immune function is mediated via GABA(A) receptors. PLoS One. 2011;6(2):e17152. doi: 10.1371/journal.pone.0017152. PubMed PMID: 21390329; PMCID: PMC3044756.

30. Xiang YY, Chen X, Li J, Wang S, Faclier G, Macdonald JF, Hogg JC, Orser BA, Lu WY. Isoflurane regulates atypical type-A gamma-aminobutyric acid receptors in alveolar type II epithelial cells. Anesthesiology. 2013;118(5):1065-75. Epub 2013/03/15. doi: 10.1097/ALN.0b013e31828e180e. PubMed PMID: 23485993.

31. Boost KA, Leipold T, Scheiermann P, Hoegl S, Sadik CD, Hofstetter C, Zwissler B. Sevoflurane and isoflurane decrease TNF-alpha-induced gene expression in human monocytic THP-1 cells: potential role of intracellular IkappaBalpha regulation. Int J Mol Med. 2009;23(5):665-71. Epub 2009/04/11. doi: 10.3892/ijmm_00000178. PubMed PMID: 19360326.

32. Lin KH, Lu WJ, Wang SH, Fong TH, Chou DS, Chang CC, Chang NC, Chiang YC, Huang SY, Sheu JR. Characteristics of endogenous gamma-aminobutyric acid (GABA) in human platelets: functional studies of a novel collagen glycoprotein VI inhibitor. J Mol Med (Berl). 2014;92(6):603-14. Epub 2014/03/15. doi: 10.1007/s00109-014-1140-7. PubMed PMID: 24626935.

33. Manne BK, Denorme F, Middleton EA, Portier I, Rowley JW, Stubben C, Petrey AC, Tolley ND, Guo L, Cody M, Weyrich AS, Yost CC, Rondina MT, Campbell RA. Platelet gene expression and function in patients with COVID-19. Blood. 2020;136(11):1317-29. Epub 2020/06/24. doi: 10.1182/blood.2020007214. PubMed PMID: 32573711; PMCID: PMC7483430.

34. Hottz ED, Azevedo-Quintanilha IG, Palhinha L, Teixeira L, Barreto EA, Pao CRR, Righy C, Franco S, Souza TML, Kurtz P, Bozza FA, Bozza PT. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with

severe COVID-19. Blood. 2020;136(11):1330-41. Epub 2020/07/18. doi: 10.1182/blood.2020007252. PubMed PMID: 32678428; PMCID: PMC7483437.

35. De Albuquerque N, Baig E, Ma X, Zhang J, He W, Rowe A, Habal M, Liu M, Shalev I, Downey GP, Gorczynski R, Butany J, Leibowitz J, Weiss SR, McGilvray ID, Phillips MJ, Fish EN, Levy GA. Murine hepatitis virus strain 1 produces a clinically relevant model of severe acute respiratory syndrome in A/J mice. J Virol. 2006;80(21):10382-94. Epub 2006/10/17. doi: 10.1128/JVI.00747-06. PubMed PMID: 17041219; PMCID: PMC1641767.

36. Khanolkar A, Hartwig SM, Haag BA, Meyerholz DK, Epping LL, Haring JS, Varga SM, Harty JT. Protective and pathologic roles of the immune response to mouse hepatitis virus type 1: implications for severe acute respiratory syndrome. J Virol. 2009;83(18):9258-72. Epub 2009/07/03. doi: 10.1128/JVI.00355-09. PubMed PMID: 19570864; PMCID: PMC2738266.

37. Khanolkar A, Hartwig SM, Haag BA, Meyerholz DK, Harty JT, Varga SM. Toll-like receptor 4 deficiency increases disease and mortality after mouse hepatitis virus type 1 infection of susceptible C3H mice. J Virol. 2009;83(17):8946-56. Epub 2009/06/26. doi: 10.1128/JVI.01857-08. PubMed PMID: 19553337; PMCID: PMC2738158.

38. Khanolkar A, Fulton RB, Epping LL, Pham NL, Tifrea D, Varga SM, Harty JT. T cell epitope specificity and pathogenesis of mouse hepatitis virus-1-induced disease in susceptible and resistant hosts. J Immunol. 2010;185(2):1132-41. Epub 2010/06/18. doi: 10.4049/jimmunol.0902749. PubMed PMID: 20554960; PMCID: PMC2897948.

39. Tian J, Milddleton B, Kaufman DL. GABA administration prevents severe illness and death following coronavirus infection in mice. bioRxiv. 2020. Epub 2020/10/08. doi: 10.1101/2020.10.04.325423. PubMed PMID: 33024975; PMCID: PMC7536896.

40. Leibowitz J, Kaufman G, Liu P. Coronaviruses: propagation, quantification, storage, and construction of recombinant mouse hepatitis virus. Curr Protoc Microbiol. 2011;Chapter 15:Unit 15E 1. Epub 2011/05/04. doi: 10.1002/9780471729259.mc15e01s21. PubMed PMID: 21538303; PMCID: PMC3119930.

41. Hamilton MA, Russo RC, Thurston RV. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol. 1977;11(7):714-9.

42. Jin N, Kolliputi N, Gou D, Weng T, Liu L. A novel function of ionotropic gamma-aminobutyric acid receptors involving alveolar fluid homeostasis. J Biol Chem. 2006;281(47):36012-20. Epub 2006/09/28. doi: 10.1074/jbc.M606895200. PubMed PMID: 17003036.

43. Bai D, Fang L, Xia S, Ke W, Wang J, Wu X, Fang P, Xiao S. Porcine deltacoronavirus (PDCoV) modulates calcium influx to favor viral replication. Virology. 2020;539:38-48. Epub 2019/11/02. doi: 10.1016/j.virol.2019.10.011. PubMed PMID: 31670218; PMCID: PMC7112098.

44. Kraeft SK, Chen DS, Li HP, Chen LB, Lai MM. Mouse hepatitis virus infection induces an early, transient calcium influx in mouse astrocytoma cells. Exp Cell Res. 1997;237(1):55-62. Epub 1998/01/07. doi: 10.1006/excr.1997.3768. PubMed PMID: 9417866; PMCID: PMC7133765.

45. Kim JK, Kim YS, Lee HM, Jin HS, Neupane C, Kim S, Lee SH, Min JJ, Sasai M, Jeong JH, Choe SK, Kim JM, Yamamoto M, Choy HE, Park JB, Jo EK. GABAergic signaling linked to autophagy enhances host protection against intracellular bacterial infections. Nat Commun. 2018;9(1):4184. Epub 2018/10/12. doi: 10.1038/s41467-018-06487-5. PubMed PMID: 30305619; PMCID: PMC6180030.

46. Huang S, Mao J, Wei B, Pei G. The anti-spasticity drug baclofen alleviates collagen-induced arthritis and regulates dendritic cells. J Cell Physiol. 2015;230(7):1438-47. doi: 10.1002/jcp.24884. PubMed PMID: 25556830.

47. Duthey B, Hubner A, Diehl S, Boehncke S, Pfeffer J, Boehncke WH. Anti-inflammatory effects of the GABA(B) receptor agonist baclofen in allergic contact dermatitis. Exp Dermatol. 2010;19(7):661-6. doi: 10.1111/j.1600-0625.2010.01076.x. PubMed PMID: 20201957.

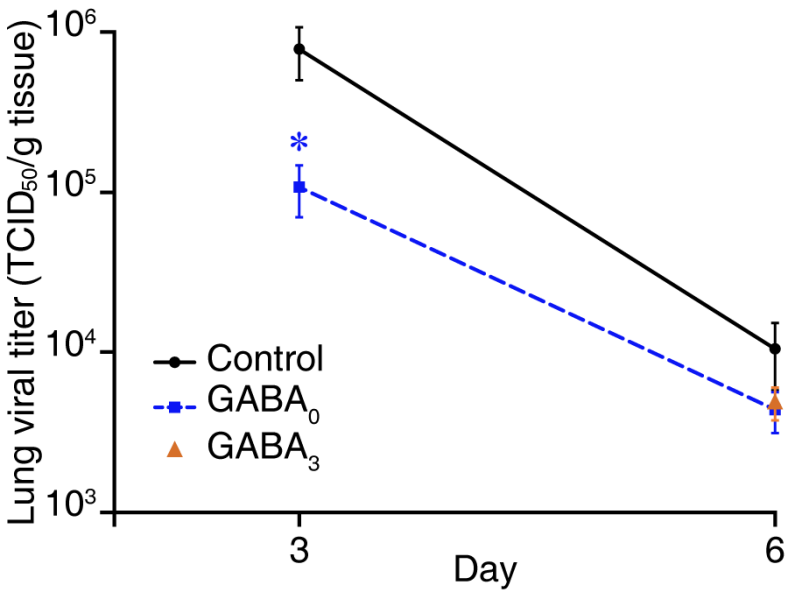

Figure 1. GABA treatment reduces viral replication in MHV-1 infected mice. Mice were inoculated with MHV-1 and placed on plain water (control) or water containing GABA immediately ($GABA_0$) or 3-days post-infection ($GABA_3$). Kinetics of MHV-1 replication in the lungs**.** A/J mice were inoculated with MHV-1 (5×10^3 PFU) and given plain drinking water, or water containing GABA, and 3 or 6 days later their lungs were harvested for determination of viral load. Concurrently, another group of MHV-1 inoculated mice was given water containing GABA beginning at 3 days post-infection and the viral load in their lungs was determined 6 days post-infection. The data shown are the mean $TCID_{50}$/g of lung tissue ±SEM at the indicated days. $GABA_0$ mice (blue square symbol) received GABA immediately after inoculation and $GABA_3$ mice (orange triangle symbol) received GABA beginning three days post-infection. N=5 mice per group at each time point. *$p<0.05$ by Student's t-test.

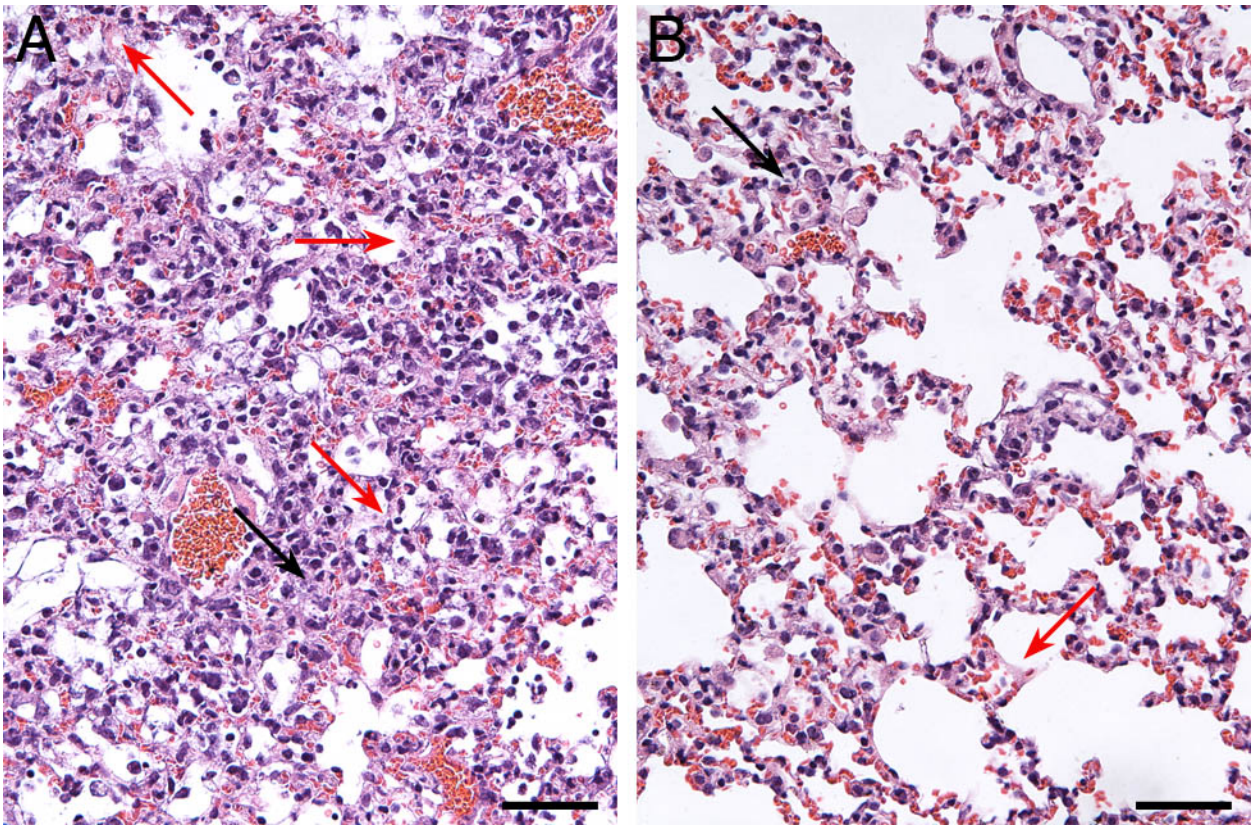

Figure 2. Histopathological features in the lungs of untreated and GABA-treated mice six days post-MHV-1 infection. Images are representative images of H&E stained lung sections from A) untreated mice and B) GABA-treated (beginning immediately following inoculation) mice six days post-infection. Red arrows point to hyaline-like membranes and black arrows indicate local consolidation. Scale bar is 50 um.