Summer 8-9-2021

Roles of a Bradykinin Storm and a Cytokine Storm in Covid-19 Cases

Nouha H. Odeh
Wayne State University, gg7297@wayne.edu

Roles of a Bradykinin Storm and a Cytokine Storm in Covid-19 Cases

Nouha Odeh

Honors Thesis

Dr. Joy Alcedo

SUMMARY

The human body's general inflammatory response, orchestrated by molecular signals, functions in both adaptive and innate immunity, allowing the body to isolate injurious agents. Unchecked, it can evolve into storms where the signals for adaptive and innate immunity are produced in excessive amounts, resulting in a severe inflammatory response. It is critical to understand how these signals are induced and ultimately stopped, since too little or too much of an inflammatory response can lead to diseased states. In light of recent events, it is imperative to understand the key inflammatory response elements that appear to be the source of more severe ailments resulting from a SARS-CoV-2 viral infection, known as Covid-19, to mitigate the effects of the pandemic. The more severe cases of Covid-19 are characterized by a severe inflammatory response resulting in tissue damage. Two possible culprits are a bradykinin storm or an interleukin-6 (IL-6)-mediated cytokine storm, where in both systems, the release of certain signals subsequently signal the production of even more of the same signal, resulting in a vicious positive feedback loop (2, 10, 21). Recent evidence points towards bradykinin, a substrate of the angiotensin-converting enzyme (ACE), as being responsible for the severe Covid-19 inflammatory response (1-4). This is because the SARS-CoV-2 virus targets ACE, a cell surface protein directly involved in bradykinin metabolism and the renin-angiotensin system (10). Bradykinin storm symptoms align similarly with what is seen in Covid-19 infection (3). **I hypothesize based on SARS-CoV-2's direct interference with the renin-angiotensin pathway, that it is the bradykinin storm responsible for the more severe complications of Covid-19, as opposed to a cytokine storm. However, isolating one storm over the other has yet to be tested. I aim to determine which storm induces the severe responses of Covid-19, such as tissue damage, hypoxemia, and fluid buildup.** To determine the nature of the storm

that induces the severe responses of Covid-19, I will test how Actemra, a cytokine IL-6 inhibitor, and icatibant, a bradykinin inhibitor, prevent such responses in a Covid-19 mouse model. The possibility exists that the systems work together, where one system induces the other or they act in parallel.

Specific Aim 1: **Is the bradykinin storm solely responsible for the severe symptoms resulting from the overactive inflammatory responses in Covid-19 patients?**

I will have four groups of humanized transgenic mouse K18-hACE2, which expresses the same human ACE receptor previously used when studying the first SARS outbreak in 2003 (5). I will infect the transgenic mouse with SARS-CoV-2, which will allow us to observe the symptoms found in patients with chronic infections, without using human patients. Previous studies have indicated that this mutated mice exhibits similar symptoms as humans upon infection (5). I will study four groups of these mice: group 1 treated with the bradykinin inhibitor; group 2 treated with the IL-6 inhibitor; group 3 given both inhibitors to test whether both pathways play a role; and group 4 is the control. I will be testing which drug will be more effective in treating the symptoms observed in the groups.

Specific Aim 2: **Does a bradykinin storm lead to a cytokine storm, or the opposite, if both systems play a role?**

If group 3 of the K18-hACE2 mice from Aim 1 have alleviated symptoms due to the presence of both inhibitors, then bradykinin and cytokines may induce the other or work in parallel. To test this, I will create a loss-of-function mutation and a gain-of-function mutation in K18-hACE2 mice. Based on bradykinin's direct interaction with the ACE protein, I expect that a

bradykinin storm will be responsible for triggering a cytokine storm, if what is observed in the third group of mice holds to be true.

BACKGROUND AND SIGNIFICANCE

Bradykinin and Renin-Angiotensin System

Bradykinin (BK) is a nonapeptide produced from a decapeptide and is normally found in the blood in its inactive form (6). BK is an extremely potent vasodilator and is a physiological mediator of anaphylaxis, which is caused by mast cell activation and release, following the binding of specific antigens to IgE antibodies on mast cell membranes (6). BK acts with the renin-angiotensin system to regulate blood flow (34).

The renin-angiotensin system functions in clearing renal waste and maintaining blood flow. A reduction in blood flow will release renin, which converts the hormone angiotensinogen into angiotensin 1, which in turn will be converted into the active angiotensin 2 (Fig 1; ref. 35). Angiotensin 2 acts on the peripheral arteries of the body to increase blood pressure via vasoconstriction and signals the adrenal glands to produce aldosterone and promote reabsorption of salts and water by the kidneys (Fig 1; ref. 35).

The counter to this system involves the membrane protein ACE2. ACE2 is a functional receptor expressed in the lungs, kidney, heart, and intestines (12). It consists of an extracellular N-terminal peptidase domain and a C-terminal transmembrane anchor (12). The active form of ACE2 is induced by the enzyme sheddase, which cleaves the external domain of the ACE2 protein (Fig 2). This cleaved ACE2 is released into the bloodstream, where it interacts with angiotensin 2, a vasoconstrictor (Fig 2; ref. 35). The conversion of angiotensin 2 to angiotensin

1-7, a powerful vasodilator and antioxidant, by ACE2 effectively inactivates angiotensin 2 and lowers blood pressure and aldosterone, antagonizing the renin-angiotensin system (Fig 2; ref. 35).

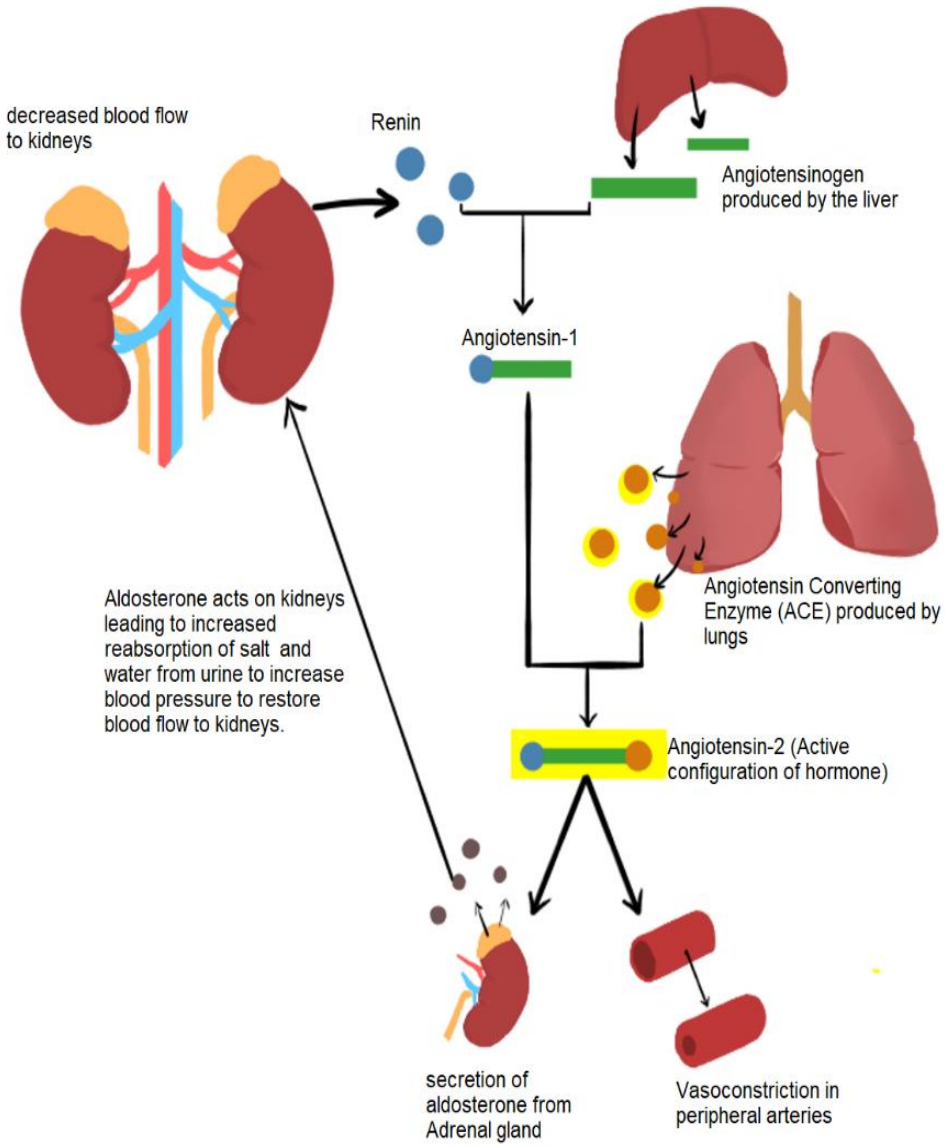

Fig 1. The renin-angiotensin system.

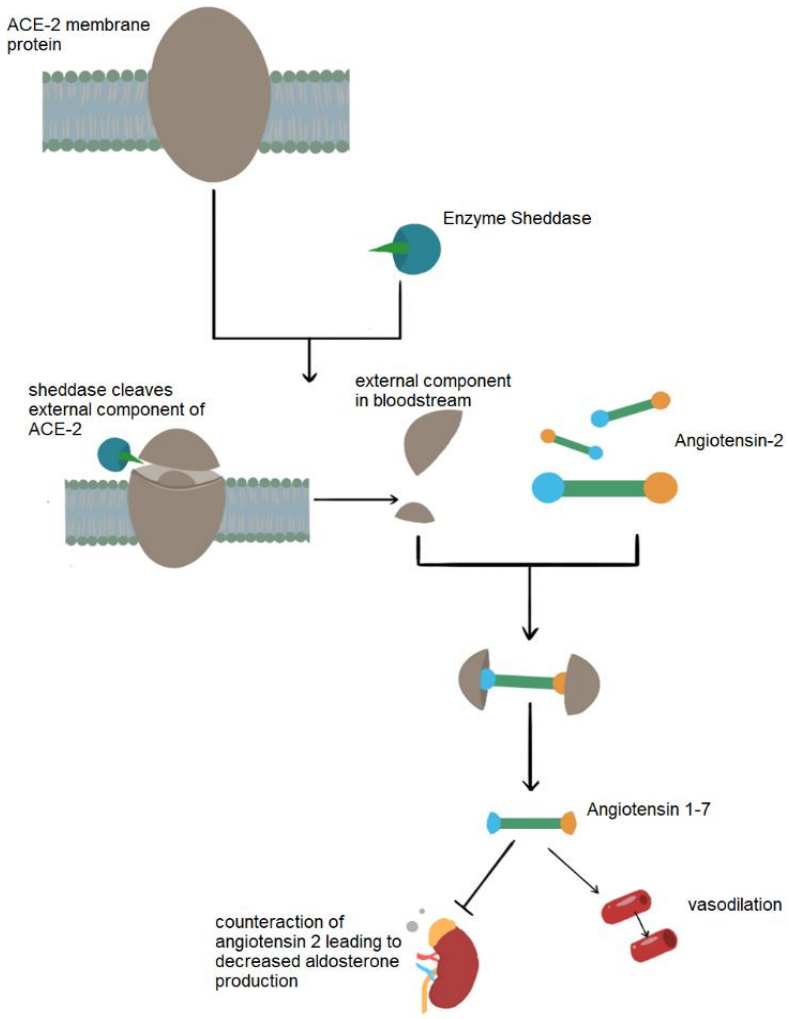

Fig 2. The activity of sheddase counters the activity of the renin-angiotensin system.

The bradykinin storm occurs when ACE2 is depleted, since this leads to increased levels of DABK, a bioactive metabolite of BK, which is associated with lung injury and inflammation (10). Depletion of ACE2 causes constant vasoconstriction that is induced by the renin-angiotensin system, which can lead to tissue damage (9, 10). High levels of DABK trigger endoplasmic reticulum stress, mitochondrial death signaling, ACE2 downregulation,

upregulation of proinflammatory genes, all of which guide the cell into apoptosis (10). Cell injury further increases the severity of the response, as it upregulates the BK B1 receptor, amplifying the DABK-mediated inflammation and damage (10, 18). This results in a positive feedback loop of inflammation and injury.

Cytokine Storm

Cytokines are a variety of proteins that are secreted by cells as signals that act on many systems of the body (17). They have autocrine, paracrine, and endocrine activities and induce a variety of responses (17). There are five major types of cytokines: interferons, which regulate innate immunity and activate antiviral properties; interleukins, which affect the growth and differentiation of leukocytes and can be proinflammatory; chemokines that control chemotaxis and leukocyte recruitment; colony-stimulating factors, which stimulate hematopoietic progenitor cell proliferation and differentiation; and lastly, tumor necrosis factors, which activate cytotoxic T lymphocytes (17). Cytokines alone are rather potent: when the signaling system produces too many, it can result in a positive feedback loop and a severe inflammatory response. This process is referred to as a cytokine storm (17). Inflammation begins at a local site and spreads throughout the body through the circulatory system. When localized within a tissue, vascular leukocytes and plasma proteins reach the extra-vascular site of injury and generate pain (17). Severe inflammation damages tissue structures and the scar tissues that form can result in organ dysfunction and long-term damage (17). In humans, a cytokine storm can result in collagen deposition in the lungs, which damage the lungs and lead to co-morbidities such as opportunistic bacterial, fungal, or viral infections and sepsis (17). Interleukin-1β (IL-1β) and interleukin-8 (IL-8) initiate the storm, appearing within the first few hours of a response and are followed by a

sustained increase in interleukin-6 (IL-6) (17). Sustained levels of IL-6 increase the severity of the storm (17).

The SARS-CoV-2 Viral infection and Covid-19

SARS-CoV-2 is a newly emerged pathogen first identified in Wuhan, Hubei Province, China, in December 2019, which causes the disease Covid-19. Initially diagnosed as a viral-induced pneumonia by clinicians, SARS-CoV-2 was eventually isolated in bronchoalveolar lavage fluid from Covid patients (2, 4, 12). Coronaviruses are serologically divided into four subfamilies: α, β, γ, and δ-coronaviruses, where the first two groups infect humans. Covid-19 was identified to be from the β coronavirus family (11). SARS-CoV-2 is a positive-sense, single-stranded RNA with a genome size of 29.9 kb and a nucleocapsid that is composed of genomic RNA and phosphorylated nucleocapsid proteins (12, 13). The genome is encapsulated by a phospholipid bilayer with two different spike proteins protruding from the membrane: a spike glycoprotein trimer common to all coronaviruses, and the hemagglutinin-esterase. SARS-CoV-2 targets the human ACE2, the functional receptor in the renin-angiotensin system. The spike proteins of SARS-CoV-2 bind to ACE2, which results in the shedding of the S1 subunit of the spike protein and a conformational change in the S2 subunit (12). This allows membrane fusion with the host cell (12). SARS-CoV-2 causes the disease Covid-19, which results in a respiratory illness characterized by fever, cough, myalgia, sputum production, heloptysis, diarrhea and dyspnea (14). Severe cases of Covid-19 can result in comorbidities like sepsis, hypoxemia, pneumonia, fluid in the lungs, and tissue damage (7, 15, 16).

The reason behind the severe symptoms of Covid-19 is hypothesized to be the direct result of a cytokine storm (2, 19, 24). The exact mechanisms of how a cytokine storm results in acute lung injury and acute respiratory distress syndrome in Covid-19 patients are unknown (25). In an analysis of 82 cases, 68 of which resulted in death, significantly higher levels of IL-6 were detected in more severe cases than in mild cases (26). Considering that sustained levels of IL-6 are associated with increasing severity of cytokine storms, this lends to the possibility that a cytokine storm is responsible for the severe cases of Covid-19.

Very recently, however, bradykinin storms are gaining attention and are also considered a possibility, since SARS-CoV-2 targets the ACE2 protein and depletes it (10). Binding of Covid-19 to the ACE2 protein prevents sheddase from binding and directly interferes with the renin-angiotensin system (10). This interference triggers increased levels of DABK, which leads to a bradykinin storm (10). The bradykinin storm is also more associated with fluid in the lungs than is the case for a cytokine storm (10). This direct interference is what has led many, including myself, to believe that a bradykinin storm is responsible for the severity of chronic SARS-CoV-2 infections (10).

It is imperative that we understand how SARS-CoV-2 interacts with the body and the mechanism by which it causes such severe damage and respiratory distress as we are still suffering through the Covid-19 pandemic. Understanding the mechanisms by which the two storms, bradykinin and cytokine, affect the body, separate or together, is crucial to mitigating the pandemic's effect, saving lives, and producing effective treatments that we presently lack. Despite this being the first coronavirus pandemic, severe epidemics of the same family have existed before and will likely exist again, especially with the emergence of new and infectious

variants of SARS-CoV-2 (27). By understanding the mechanisms through which the virus acts on our physiology, we may be able to reduce the severity of future outbreaks and save lives.

APPROACH

Specific Aim 1: Is the bradykinin storm solely responsible for the severe symptoms resulting from the overactive inflammatory responses in Covid-19 patients?

A. Rationale

SARS-CoV-2 targets the cell surface protein ACE2, inhibiting the action of the enzyme sheddase and leading to increased DABK levels (10). An increase in DABK can lead to a bradykinin storm, thus the severe inflammatory responses in Covid-19 patients (10). However, the possibility exists that a cytokine storm is the cause for the severity of Covid-19. Inhibitors of IL-6, such as Actemra, have already been approved for distribution to patients with Covid-19 (8).

B. Research Design: Viral Infection and Mice

To test the cause behind the severe Covid-19 response, I plan to use the K18-hACE2 transgenic mouse, which expresses the human ACE2 receptor, as well as the hallmark features of the human disease (28, 29). The mice are highly susceptible to SARS-CoV-1 infection and display symptoms characteristic of Covid-19 (28). Infection of SARS-CoV-1 in K18-hACE2 leads to a 14-day infection period, where the infection is almost exclusively localized to the lungs (28). I chose this model due to these similarities between SARS-CoV-1, for which the model was initially created, and SARS-CoV-2, in addition to its expression of ACE2. Previous

studies on this mouse model showed that a cytokine storm induced the inflammatory response observed in the lungs and the spleen of the mice infected with SARS-CoV-2 (29).

I will have four groups of specific-pathogen free, 4-to-5-week-old K18-hACE2 mice, where each group will have 20 mice—ten male and ten females. Three of the four groups will be infected with SARS-CoV-2 intranasally with 10^5 PFU (plaque forming units) of SARS-CoV-2 in a final volume of 50 µl following sedation. The final group, the control, will be mock-infected to ensure equal handling. I chose this concentration based on a previous study that observed an inflammatory response at this concentration (29). To prevent cross-group coprophagy and cross-infection, the groups will not interact with one another before, during, and after treatment is administered and will be given sterile water and the same type of food. After viral infection, the mice will be monitored daily for morbidity (body weight) and mortality, as well as subjected to a blood test every two days. Blood IL-6 levels and blood plasma BK levels will be analyzed and recorded for all mice.

When the mice begin to exhibit an inflammatory response, the drug treatments will begin. The first group will be given the IL-6 inhibitor Actemra, which is approved for use against a cytokine storm in Covid-19 patients, over a 14-day period. All symptoms will be recorded, as well as any changes, in addition to measurements of IL-6 and BK levels. The second group will be given icatibant, an FDA-approved BK inhibitor, over a 14-day period, where again all symptoms and changes will be recorded, as well as IL-6 and BK levels. These two groups will test if one storm is more responsible than the other for the severe Covid-19 responses; if treatment with only a BK inhibitor or only an IL-6 inhibitor proves to be effective in mitigating the inflammatory response, I can conclude that the corresponding storm is responsible for the response. The third group will also test the possibility that both storms are present at the same

time. When the third group of mice begin to exhibit an inflammatory response, they will be given both Actemra and icatibant over a 14-day period; the BK and IL-6 levels and symptoms will be recorded, just as in other groups. The final group is the control, where no treatment will be administered except for a mock infection.

C. Potential Outcomes, Caveats, and Alternative Methods

Cytokine levels have already been observed to be increased in Covid-19 patients. Thus, whether IL-6 contributes or does not contribute to the inflammatory response is not necessarily the question. However, the cause for the severe inflammatory responses of Covid-19 remain unclear. If cytokines are solely responsible for the severe inflammatory response, I would expect a decrease in IL-6 levels in the first and third group of mice, in addition to reduction of the severity of their symptoms. The same principle applies if a bradykinin storm is solely responsible: I will only observe an alleviation in the symptoms of the second and third group of animals and a decrease in BK levels. However, because the possibility exists that both could be present and potentially acting on one another, I created a third group of mice. If this is the case what I expect to observe is little to no change in the symptoms of the mice in groups one and two respectively, and an alleviation of symptoms in the third group.

However, I may also observe a mitigation of the inflammatory response with each single drug treatment, where a bradykinin storm might lead to a cytokine storm, or vice versa. I will address this outcome in Specific Aim 2.

There are also some potential concerns. The possibility exists that the mice do not generate a storm-like response or do not respond well to the drugs. If the response generated by

SARS-CoV-2 infection is too strong or does not appear at all, I will decrease or increase the concentration of SARS-CoV-2, respectively. According to previous studies, the inflammatory response necessary for this study can be produced (29). Thus, if this problem arises, changing the viral load should address this issue (29). If the mice do not respond well to one or both drugs, or if the drugs do not do what they are expected to do, I will find a different IL-6 inhibitor or a different BK inhibitor. Ideally, I can use a greater number of mice, or a quantity closer to 30-40 per group. However, the mice are in low supply and, because of the pandemic, are in high demand (28, 29).

Specific Aim 2: Does a bradykinin storm lead to a cytokine storm, or the opposite, if both systems play a role?

A. Rationale:

Understanding how one pathway functions and potentially affects the other is imperative in understanding the roles of BK and IL-6 in generating the observed inflammatory response in Covid-19 patients. To test this, single mutations and double mutations will be created and the resulting phenotypes will be compared post infection.

B. Research Design

First I will create single mutants using genetic knockouts. These will be a series of mutations to create gain-of-function mutations and loss-of-function mutations. To create a BK loss of function, I will delete the BK gene using a CRISPR/Cas9 knockout plasmid that targets

the BK gene, so that cells can no longer express BK (30, 36). To generate a BK gain of function, I will induce a bradykinin storm, which can occur when ACE2 is depleted in an organism, since it leads to increased levels of DABK (10). Thus, I will use ACE2-deficient mice, the same variety used in previous studies of the renin-angiotensin system, to induce overexpression of BK (31). Both loss-of-function and gain-of-function models will be bred for homozygosity.

To create an IL-6 loss-of-function mutation, I will use the transgenic mouse IL6-DIO-KO, which has lost IL-6 expression (33). To induce an IL-6 gain-of-function, I will use a transgenic mouse that use the Clara cell 10-kDa promoter to drive overexpression of IL-6 (32). I will also create double mutants, combining loss-of-function and gain-of-function mutations in BK and IL-6 in these mouse strains.

I will then infect the mutant mouse strains with SARS-CoV-2 and record symptoms daily, using the same procedure as described in the first specific aim.

C. Potential Outcomes, Caveats, and Alternative Methods.

A major concern rests with the BK loss-of-function mutant or in the double mutants, for example, the mutants expressing loss-of-function mutations in both IL-6 and BK. There is a possibility that these mice may not be viable or may present a multitude of health issues and might not survive SARS-CoV-2 infection long enough for me to observe responses. The same could be true for the mutants expressing both gain-of-function mutations. If this were to happen, I would have to find a way to create a reduction-of-function mutation, as opposed to a complete loss-of-function mutation, such as introducing siRNAs against BK to create a reduction-of-function mutation without completely deleting the gene (37).

Another potential concern is that ACE2-depleted mice might not cause a BK storm. A reduction of ACE2 leads to increased DABK, but there is no guarantee that this alone will cause a BK storm in transgenic mice. If this were to happen, an alternative approach would be to couple this mutation with another mutation that causes overexpression of BK receptors (38, 39).

If the phenotype of the double mutants matches one of the single mutations, I can conclude the epistasis between the pathways. If the phenotype of the double mutants does not match any of the single mutants, then the two pathways might work in parallel. While the exact mechanism might not be known on how these two pathways act in parallel, my proposed experiments will provide insight on the interaction between the two pathways.

CONCLUSION

As of July 2021, almost 200 million people have contracted Covid-19 and over 4 million have died (40). While the fatality rate has certainly decreased over time, the effects of the pandemic changed daily life drastically for many (41). The long-term effects of the disease remain largely unknown because of its recent occurrence, and it becomes increasingly imperative that the mechanisms of human immunity are understood to prevent the deaths of millions. This was not the first pandemic that we have encountered and will certainly not be the last. Understanding the body's adaptive and innate immune responses will allow us to create treatments for future epidemics and pandemics. This may also allow us to predict the way future strains might affect our bodies before they bloom into devastating pandemics. A further understanding of how bradykinins and cytokines function together to create the inflammatory responses observed as a result of SARS-CoV-2 infection and other diseases is also absolutely critical in developing effective treatments for these diseases. Knowing how both pathways potentially interact with one

another would allow us to prevent prolonged tissue damage in the instance of a bradykinin or a cytokine storm. Besides Covid-19, my study should also yield insight into the study of other diseases that result in severe inflammatory responses caused by these molecular signals.

Literature Citations

1. Pérez, E. G., Cayuelas, J. M. A., Peñalver, M. N., & Martínez, A. M. (2021). Elevated baseline renin levels as a possible cause of worse prognosis of COVID-19 in patients with heart disease. *Revista Espanola De Cardiologia (English Ed.)*, *74*(2), 203.
2. Mahmudpour, M., Roozbeh, J., Keshavarz, M., Farrokhi, S., & Nabipour, I. (2020). COVID-19 cytokine storm: The anger of inflammation. *Cytokine*, 155151.
3. Pagliaro, P., & Penna, C. (2020). ACE/ACE2 ratio: a key also in 2019 coronavirus disease (Covid-19)?. *Frontiers in medicine*, *7*.
4. Tolouian, R., Vahed, S. Z., Ghiyasvand, S., Tolouian, A., & Ardalan, M. (2020). COVID-19 interactions with angiotensin-converting enzyme 2 (ACE2) and the kinin system; looking at a potential treatment. *Journal of Renal Injury Prevention*, *9*(2), e19-e19.
5. Moreau, G. B., Burgess, S. L., Sturek, J. M., Donlan, A. N., Petri Jr, W. A., & Mann, B. J. (2020). Evaluation of K18-hACE2 mice as a model of SARS-CoV-2 infection. *The American Journal of Tropical Medicine and Hygiene*, *103*(3), 1215-1219.
6. Bradykinin. (n.d.) *Farlex Partner Medical Dictionary*. (2012). Retrieved February 4 2021 from https://medical-dictionary.thefreedictionary.com/bradykinin
7. Brito-Azevedo, A., Pinto, E. C., de Cata Preta Corrêa, G. A., & Bouskela, E. (2021). SARS-CoV-2 infection causes pulmonary shunt by vasodilatation. *Journal of medical virology*, *93*(1), 573-575.
8. Jean, S. S., & Hsueh, P. R. (2020). Old and re-purposed drugs for the treatment of COVID-19. *Expert Review of Anti-infective Therapy*, *18*(9), 843-847.
9. McCarthy, C. G., Wilczynski, S., Wenceslau, C. F., & Webb, R. C. (2020). A new storm on the horizon in COVID-19: Bradykinin-induced vascular complications. *Vascular Pharmacology*.
10. Roche, J. A., & Roche, R. (2020). A hypothesized role for dysregulated bradykinin signaling in COVID-19 respiratory complications. *The FASEB Journal*, *34*(6), 7265-7269.
11. Samudrala, P. K., Kumar, P., Choudhary, K., Thakur, N., Wadekar, G. S., Dayaramani, R., ... & Alexander, A. (2020). Virology, pathogenesis, diagnosis and in-line treatment of COVID-19. *European Journal of Pharmacology*, *883*, 173375.
12. Jin, Y., Yang, H., Ji, W., Wu, W., Chen, S., Zhang, W., & Duan, G. (2020). Virology, epidemiology, pathogenesis, and control of COVID-19. *Viruses*, *12*(4), 372.

13. National Microbiology Data Center. Available online: **http://nmdc.cn/coronavirus** (accessed on 3 March 2020).
14. Novel Coronavirus Pneumonia Emergency Response Epidemiology Team. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China. *Zhonghualiuxingbingxuezazhi* **2020**, *41*, 145–151.
15. Huang, H., Cai, S., Li, Y., Li, Y., Fan, Y., Li, L., ... & Deng, X. (2020). Prognostic factors for COVID-19 pneumonia progression to severe symptoms based on earlier clinical features: A retrospective analysis. *Frontiers in medicine*, *7*, 643.
16. Kamal, M., Abo Omirah, M., Hussein, A., & Saeed, H. (2020). Assessment and characterisation of post-COVID-19 manifestations. *International Journal of Clinical Practice*, e13746.
17. Tisoncik, J. R., Korth, M. J., Simmons, C. P., Farrar, J., Martin, T. R., & Katze, M. G. (2012). Into the eye of the cytokine storm. *Microbiology and Molecular Biology Reviews*, *76*(1), 16-32.
18. Leeb-Lundberg, L. F., Marceau, F., Müller-Esterl, W., Pettibone, D. J., & Zuraw, B. L. (2005). International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences. *Pharmacological reviews*, *57*(1), 27-77.
19. Hu, B., Huang, S., & Yin, L. (2021). The cytokine storm and COVID-19. *Journal of medical virology*, *93*(1), 250-256.
20. Ye, Q., Wang, B., & Mao, J. (2020). Cytokine storm in COVID-19 and treatment. *Journal of Infection*.
21. Tang, Y., Liu, J., Zhang, D., Xu, Z., Ji, J., & Wen, C. (2020). Cytokine storm in COVID-19: the current evidence and treatment strategies. *Frontiers in immunology*, *11*, 1708.
22. Sun, X., Wang, T., Cai, D., Hu, Z., Liao, H., Zhi, L., ... & Wang, A. (2020). Cytokine storm intervention in the early stages of COVID-19 pneumonia. *Cytokine & growth factor reviews*, *53*, 38-42.
23. Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., & Manson, J. J. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *The lancet*, *395*(10229), 1033-1034.
24. Wright, D. J. (2021). Prevention of the cytokine storm in COVID-19. *The Lancet Infectious Diseases*, *21*(1), 25-26.

25. Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R., & Salem, R. (2020). The COVID-19 cytokine storm; what we know so far. *Frontiers in immunology*, *11*, 1446.
26. Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R., & Salem, R. (2020). The COVID-19 cytokine storm; what we know so far. *Frontiers in immunology*, *11*, 1446.
27. Koyama, T., Weeraratne, D., Snowdon, J. L., & Parida, L. (2020). Emergence of drift variants that may affect COVID-19 vaccine development and antibody treatment. *Pathogens*, *9*(5), 324.
28. Johansen, M. D., Irving, A., Montagutelli, X., Tate, M. D., Rudloff, I., Nold, M. F., ... & Hansbro, P. M. (2020). Animal and translational models of SARS-CoV-2 infection and COVID-19. *Mucosal immunology*, *13*(6), 877-891.
29. Oladunni, F. S. et al. Lethality of SARS-CoV-2 infection in K18 human angiotensin converting enzyme 2 transgenic mice. Preprint at https://doi.org/10.1101/2020.07.18.210179 (2020).
30. Bradykinin CRISPR Knockout and Activation Products (h). (n.d.). Retrieved May 15, 2021, from https://www.scbt.com/p/bradykinin-crispr-knockout-and-activation-products-h
31. Wang, J., Kaplan, N., Wysocki, J., Yang, W., Lu, K., Peng, H., ... & Lavker, R. M. (2020). The ACE2-deficient mouse: A model for a cytokine storm-driven inflammation. *The FASEB Journal*, *34*(8), 10505-10515.
32. Gomez-Arroyo, J., Saleem, S. J., Mizuno, S., Syed, A. A., Bogaard, H. J., Abbate, A., ... & Voelkel, N. F. (2012). A brief overview of mouse models of pulmonary arterial hypertension: problems and prospects. *Ame: rican Journal of Physiology-Lung Cellular and Molecular Physiology*, *302*(10), L977-L991.
33. Sanchis, P., Fernández-Gayol, O., Comes, G., Aguilar, K., Escrig, A., Giralt, M., ... & Hidalgo, J. (2020). A new mouse model to study restoration of interleukin-6 (IL-6) expression in a Cre-dependent manner: microglial IL-6 regulation of experimental autoimmune encephalomyelitis. *Journal of Neuroinflammation*, *17*(1), 1-17.
34. Hornig, B., & Drexler, H. (1997). Endothelial function and bradykinin in humans. *Drugs*, *54*(5), 42-47.
35. Santos, P. C. J. L., Krieger, J. E., & Pereira, A. C. (2012). Renin–angiotensin system, hypertension, and chronic kidney disease: pharmacogenetic implications. *Journal of pharmacological sciences*, *120*(2), 77-88.
36. Ran, F. A., Hsu, P. D., Wright, J., Agarwala, V., Scott, D. A., & Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. *Nature protocols*, *8*(11), 2281-2308.

37. Doench, J. G., Petersen, C. P., & Sharp, P. A. (2003). siRNAs can function as miRNAs. *Genes & development*, *17*(4), 438-442.

38. Ni, A., Yin, H., Agata, J., Yang, Z., Chao, L., & Chao, J. (2003). Overexpression of kinin B1 receptors induces hypertensive response to des-Arg9-bradykinin and susceptibility to inflammation. *Journal of Biological Chemistry*, *278*(1), 219-225.

39. Wang, D. Z., Chao, L., & Chao, J. (1997). Hypotension in transgenic mice overexpressing human bradykinin B2 receptor. *Hypertension*, *29*(1), 488-493.

40. Coronavirus Cases:. (n.d.). Retrieved July 30, 2021, from https://www.worldometers.info/coronavirus/

41. Coronavirus (COVID-19) Mortality Rate. (n.d.). Retrieved July 30, 2021, from https://www.worldometers.info/coronavirus/coronavirus-death-rate/