International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 9, Issue 9 -2022

Review Article

DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.09.003

# Affinity Toward Mucormycosis in COVID-19 Patients

**Subhendu Sekhar Bag*,[1,2] and Sayantan Sinha[2]**

[1]Chemical Biology/Genomics Laboratory, Department of Chemistry, and
[2]Center for the Environment, Indian Institute of Technology Guwahati, Guwahati-781039.
E-mail: *ssbag75@iitg.ac.in*

## Abstract

The immunological responses under COVID-19 and Mucormycosis diseased conditions are reported herein. In addition, the affinity of developing Mucormycosis in SARS-CoV-2 infected/recovered patients is correlated with their immunochemistry. Thus, this study would help understand T cells' importance and open up a new avenue for designing an alternative vaccine to mitigate both COVID-19 and Mucormycosis simultaneously.

**Keywords:** COVID-19, Mucormycosis, T cells, immunochemistry.

## Highlights:

- COVID-19 Host cell Immune Response|
- Opportunistic *Rizopus oryzae* Infection in COVID-19 patients|
- T Cell Response in COVID-19 and Mucormicosis|
- Scope for an Alternate and Effective Vaccine|
- T Cell-based Vaccine: Need of the Hour|

## Introduction

### COVID-19 Host Cell Immune Response

***Immune Response:*** SARS-CoV-2 infects human host cells by endocytosis modulated through the interaction of the viral surface spiked glycoproteins with specific human Angiotensin-converting enzyme 2 *(hACE2)* receptors on the apical surface of human epithelial cells. As a result, the hosts' innate and adaptive immune systems immediately recognizes the whole virus or the expressed epitopes as an outside threat (antigen). In the first step, the Toll-like receptors (TLR3, TLR7 and TLS8) on the surface of the host immune cells, such as the macrophages and dendritic cells, recognizes the viral antigen and elicits the production of interferon (IFN).

Afterwards, the type 1 interferon (IFN-α/β) starts priming the $CD8^+$ T cells by activating the Antigen Presenting Cells (APCs). Furthermore, apart from accelerating the activation of adaptive response, IFN also signals the innate immune system to respond.

As a result of the innate immune response, B cells immediately generate neutralizing antibodies against the N protein of the virus which develops high immunogenicity due to the absence of glycosylation sites.[1] On the other hand, the antibody response of host cell specific to viral S protein is generated 4-8 days from initial infection/onset of symptoms. In a recent case study with 16 SARS-CoV-2 patients, anti N protein IgG and anti-S-RBD protein IgG hve been detected in 15 and 16 patients, respectively. On the other hand, S-RBD protein IgM and N protein IgM hve also been found in samples of 14 and 15 patients, respectively.[2] Another study reported that several patients have been detected with an elevated level of IgG and IgM in the collected blood specimens. The results have shown that a lower response of IgG corresponds to faster viral clearance, while a robust response indicates the severity of disease prognosis.[3] It is noteworthy to mention that the resistance of SARS-CoV-2 against the antibodies has recently emerged as a critical concern.[4] Therefore, scientists are investigating the other immune factors that can potentially interact with virus-laden cells. Such factors might target and eliminate the virus-laden cells. As a result of tremendous research effort, scientists now believe that T cells would provide an immunity booster and help fight against SARS-CoV-2 infection. Therefore, understanding the role of T cells is of paramount importance to mitigate COVID-19.

***Antigen Presentation:*** The viral peptide epitopes (N and S protein) are presented by MHC 1 to cytotoxic $CD8^+$ T cells and MHC II molecules to $CD4^+$ helper T cells. Previous studies on SARS-CoV identified 14 epitopes localized in ORF3, out of which S proteins were found to elicit the T cell response. While S protein was the primary epitope for activation of cytotoxic T cells in SARS-CoV, both N and S proteins and few M/E peptides served as epitopes for activating the response of MERS-CoV. A recent study[6] with recovering COVID-19 patients reported a strong immune response elicited by CD4+ T cells against S, N and M protein epitopes of the virus. Further, the study correlated the effect of the adaptive immune response on the humoral immune system. The outcome indicated an increased titer of specific IgG and IgA antibody against S-RBD viral protein. Further, it was also observed that along with $CD4^+$ T cells, the $CD8^+$ cytotoxic T cells have also been activated by the viral S, N and M epitopes. This unique response of the T cells was not only limited to the COVID patients but also found in 60-70% of non-infected individuals.[6] A detailed analysis revealed that these non-infected individuals have pre-generated $CD4^+$ helper T cells. This result signifies that they might have come in contact with the virus at some point and developed immunity against SARS-CoV-2.

***Role of Memory T cell*:** In the case of re-infection, the role of memory T cells become most prominent, which act as a game-changer to save individual's life. It has also been reported that reactivation/re-stimulation of CD4+ memory T cells results in signalling cascades generating cytokines. These cytokines activate the B cell antibodies and other immune cells. Similarly, the memory cytotoxic T cells initiate activation of $CD8^+$ T cells, which directly attacks and kills the pathogen.[5,6] A previous study on recovered SARS-CoV patients confirmed that memory CD4+ and CD8+ cells could initiate an immune response in between age groups of 3 months to 6 years.[7] In another case study[8] with 23 recovered SARS-CoV patients, it was observed that 60% of the patients showed a higher population of viral S epitope-specific memory T cells. Interestingly in those cases, the population of B memory cells was found to be minimal. As the immunogenic response of SARS-CoV-2 is similar to SARS-CoV[1], a similar trend is expected in the case of SARS-CoV-2 infected patients.

However, the generation of adaptive immune cells (T cells) in COVID-19 patients is often overshadowed by the excessive production of cytokines and chemokines due to the compromised early innate immune response. This cytokine storm is a major cause for multi-organ failure in COVID-19 patients leading to death. A recent study[9a] reported that the lack of T cells resulted in an increased level of TNF and IFN-γ proinflammatory cytokines when a T cell-deficient matured nude mice was compromised with a sublethal dose of MHV-A59 coronavirus. They inferred that this excessive increase in the cytokines leads to the death of the infected mice. Another study on COVID-19 patients reported that the primary reason for the elevated cytokine storm resulting in multi-organ and tissue damage resulted from a decreased frequency of T cells.[9b] Therefore, T cells have an extremely crucial role in regulating/controlling the balance of cytokine generation in the infected host and also saving the immunocompromised host from the adverse effects of the cytokine storm.

Furthermore, the analysis of compromised innate immunity in COVID-19 patients reflected that adaptive immunity has a significant role in tackling the disease. However, there is another threat in COVID-19 patients due to their immune-compromised condition. These patients are often vulnerable to various secondary infections from fungus, bacteria and other pathogens. One such opportunistic secondary infection is seen recently in COVID-19 patients from the infection of a particular fungus, *Rhizopus oryzae*. The disease is known as Mucormycosis. In India, over 8000 cases of Mucormycosis as a secondary infection in COVID-19 patients have been registered. The Indian government has recently declared Mucormycosis an epidemic.

## Opportunistic *Rhizopus oryzae* Infection in COVID-19 patients

*Mucormycosis:* Mucormycosis, or colloquially referred to as Black Fungi disease, is caused due to an infection by the filamentous sporulating fungi belong to the Mucorales order.[10] Around 70% of cases, mucormycosis has resulted from the infection of *R. oryzae*. The fungi are commonly present in the environment and invade the host through nostrils on inhalation of fungal spores. The fungi can also invade through cuts or tears in the skin. Mucormycosis disease can occur in children and adults suffering from severe or uncontrolled diabetes, hemochromatosis, and immune-compromised conditions under diseases like HIV, cancer, etc.

In general, the *Mucorales* species targets the sinus and impairs the nasal, cerebral and ocular functioning. This particular disease prognosis is known as Rhinocerebral Mucormycosis or Zygomycosis. The clinical symptoms of the disease are nasal cellulitis, reddening of the nasal bridge, and gradual reddening of the cheeks. In due course of the infection, the cells start dying, and a black patch is observed around the eyes and nose. Visible black Escher on nasal mucosa or palatine mucosa with profuse bleeding from the nose is seen in severe cases. Ulceration can be detected in the palate, and ophthalmoplegia can be observed. If the cerebral infection has started spreading, the patients' vascular system becomes compromised. This leads to a coma or cerebral stroke resulting from paralysis of second to seventh cranial nerves. Few associated eye-related symptoms like nystagmus, conjunctival chemosis, and blown pupil can also be observed in patients. Another related prognosis has resulted from the excessive sporulation of the fungi in the lungs. This particular condition is known as Pulmonary Mucormycosis, resulting in impairment of alveolar functioning. Initial symptoms arise in the form of breathing troubles, coughing, headache, coughing out blood and fever. Diagnosis of the disease is made through the collection of pulmonary fluid, chest X-ray and tissue biopsy.

In India, the cases of Mucormycosis are reported as a secondary infection in patients suffering or who have recovered from COVID-19. This is because COVID patients have compromised innate immune system. Further, as a treatment procedure, steroids are often used in acute cases, which again weaken the natural immune response.

Therefore this makes the COVID patients vulnerable to infection from the Black Fungi. Some instances of Mucormycosis have also been reported in recovered COVID patients. The prognosis is new, but most likely, in these cases, the innate immune system fails to tackle the fungi. At present, the only treatment avenue is stopping the immune-modulatory drugs or steroids. However, managing acute COVID patients becomes a challenge in this process. Patients suffering from Mucormycosis are monitored with saline and then injected with Amphotericin B or other antifungal drugs. The standard course of treatment has been decided by medical practitioners for at least 4-6 weeks.

When the fungi infect an average healthy individual, the pathogen confronts the first line of innate immune defence comprised of the leukocytes and B cells. The fungal pathogen is recognized by the TLR2 present ubiquitously. On interaction with the pathogen, the TLR immediately triggers the generation of cytokines and chemokines such as IL-8, IL1α/β, CXCL1/2, TNF and GM-CSF. These molecules immediately signal for the recruitment of the polymorphonuclear leukocytes or neutrophils. In this process, the pathogen is engulfed through phagocytosis. Neutrophils kill the fungal pathogen by producing superoxide with the help of the NADPH oxidase enzyme.[11]

However, few recent studies have reported that some patients recovering from cancer with a normal neutrophil count acquired the secondary infection from *R.oryzae*. Hence innate immune system may be less effective against *Mucorales* species in the case of recovered or recovering patients.[11] In the same context, another group has shown the presence of a persistant level of specific anti-Mucorales-hyphae T cells in recovering patients that carried out their activity until the elimination of the infection.[12-14] Few other studies have also reported the presence of specific T cells in the peripheral blood in patients suffering and/or recovered from Mucormycosis.[15, 16]

Therefore, it is obvious that T cells can play a crucial role in fighting Mucormycosis, in absence of activted innate immune system. It is now clear that the current COVID-19 disease and the treatment medications such as steroids ultimately leads to compromised/weakened immune system of the host. As a result, the COVID-19 patients become vulnerable to secondary infections like Mucormycosis. However, the treatment planning and medications for curing Mucormycosis are completely different from COVID-19. This situation has created a dilemma. Therefore, to overcome the crisis, the need of the hour is to develop a particular drug/vaccine or a combination that can provide a cure to both the diseases simultaneously.

## T Cell Response in COVID-19 and Mucormicosis

It is now very much clear that with the primary infection from SARS-CoV-2, the innate immune response is completely shattered. As a result, generation of cytokine storm takes place immediately, harming the host in many ways, such as multi-organ failure or even death. Moreover, all the above discussions have clearly suggested that activating the adaptive immune response instantly at the onset of infections is the best possible way to restrengthen the hosts' defence mechanism and calm the cytokine storm to fight the primary as well as the secondary infections simultaneously.

Furthermore, it is now well understood that the post infections of both SARS-CoV-2 and Mucorales have led to the generation of a significant population of $CD4^+$ and $CD8^+$ T cells and memory T cells in the host. These T cells have a profound effect of recovering the patients from such pathogenic infections in various ways, such as by targeting and killing the pathogens and protecting host from the adverse impact of cytokines.

Hence, we envision that the application of host T cells would be a promising possibility for the realization of a specific drug/vaccine to tackle

both COVID-19 and Mucormycosis. We have already discussed that the T cells and memory T cells play a crucial role in tackling both the COVID-19 and Mucormycosis diseases. Therefore, it is of utmost importance that the pharmaceutical companies and scientists to come forward and to consider the designing of T cell-based vaccine/medicine towards complete remission of the disease manifestations.

## Scope for an Alternate and Effective Vaccine

To tackle COVID-19 pandemic, vaccination process is continuing at a fast pace across the world. At present, a total of twelve vaccines such as Moderna Spikevax, Pfizer-BioNTech Comirnaty, Sputnik V, Oxford AstraZeneca-Covishield, BBBP-CorV, CoronaVac, Novavax, J&J Jcovden Covaxin, Valneva VLA2001 and COVOVAX have been permitted to be applied for medical practice and mass vaccination. Based on the availability and considering all possible constrain, various countries are applying different vaccines with an overall beneficial impact on individual's health.

In India, as of 04$^{th}$ September 2022,2, 13, 52,74,945 citizens have been vaccinated. Indian made Covaxin and Astrazeneca-Oxford Covishield are the major vaccines that are administered to Indian citizens. Vaccines that are currently administered throughout the world are either attenuated CoV-2 virus or genetically modified adenovirus or CoV-2 mRNA. All the vaccines are still undergoing the final stages of clinical trials. A fascinating observation has come up that even after getting vaccinated, several cases of re-infection with SARS-CoV-2 are reported worldwide. Hence this suggests that the efficacy of the vaccines is fading against various mutated strains of SARS-CoV-2.

Therefore, scientists and vaccine developing companies worldwide have now focused on exploring alternative avenues, mainly focusing on the immunogenic response for the development of a vaccine that can become more effective against wide viral mutations. Additionally, with the onset of secondary infection from *Micorales*, it has become an utmost need to develop a strategy to fight both COVID-19 and Mucormycosis simultaneously with a single vaccine shot if possible. The impact of T cells has now attracted scientists in their recent research in vaccine development.

We have already discussed how adaptive immunity plays an essential and major role in various SARS infections. Further discussed that during SARS-CoV-2 infection alongside the production of B cell antibodies, the host immune system generates an army of cells that can specifically target and destroy the virus. Thus, recent research has shown that memory T cells and the T cells can become a game-changer in this endeavour. Among these cells, a significant population of cytotoxic CD8+ T cells can recognize and strike down pathogens. Moreover, the cytotoxic killer cells are assisted by the Helper T cells, which produce a cascade of signals for activating various immune functions.

Although the infection cannot be prevented by the T cells as they get activated and start performing their activity only after a pathogen has invaded the host, they are the key players in sweeping the pathogen and stopping the infection. A recent study[17] has shown that T cells are more robust than antibodies when confronted with mutated viral variants. They have reported that SARS-CoV-2 infected patients produced specific T cells, which are able to target 15 different protein fragments of the CoV-2 virus. It was also observed that the protein fragments recognized by the T cells as their target are of various kinds for all the clinical cases.

Therefore, this study shows that large and varied families of specific T cells have been produced upon infection, compromising the virus to get recognized. However, this phenomenon is not seen in the case of antibodies.[17] Study had shown a protective response when a specific $CD4^{+}$ memory T cells were injected as a vaccine through the subcutaneous route of hCoV infected mice. The memory T cells could generate IFN-γ

which activated $CD8^+$ killer T cells and ultimately, the killer T cells resulted in complete viral clearance.[18]

Thus, it is clear that the T cells have a significant impact in tackling COVID-19 and the associated secondary infection at a time. The role of T cell in neutralizing SARS-CoV-2 and *R. oryzea* is shown in **Figure 1**. Thus, looking at the possibility of utilising T cells towards mitigation of the COVID-19, many vaccine developers have proposed to synthesize next-generation vaccines that would activate specific T cells in the host. Antibodies mainly detect various proteins on the surface of the CoV-2 virus. Such as the spiked protein has been a significant target for the B cell antibodies. However, the particular S protein is non-conserved and gets mutated frequently, increasing the possibility that antibodies cannot recognize or act against variable mutants of CoV-2. In contrast, T cells target all the proteins which are expressed by the viral genome inside the host cells. The mRNA of CoV-2 undergoes the replication-transcription process to express few stable proteins against which specific T cells generate a response to recognize the virus. Therefore, the host T-cell response in detecting and clearing the virus, tackling secondary infection and balancing the adverse effect of cytokines has opened up a new avenue in vaccine research.

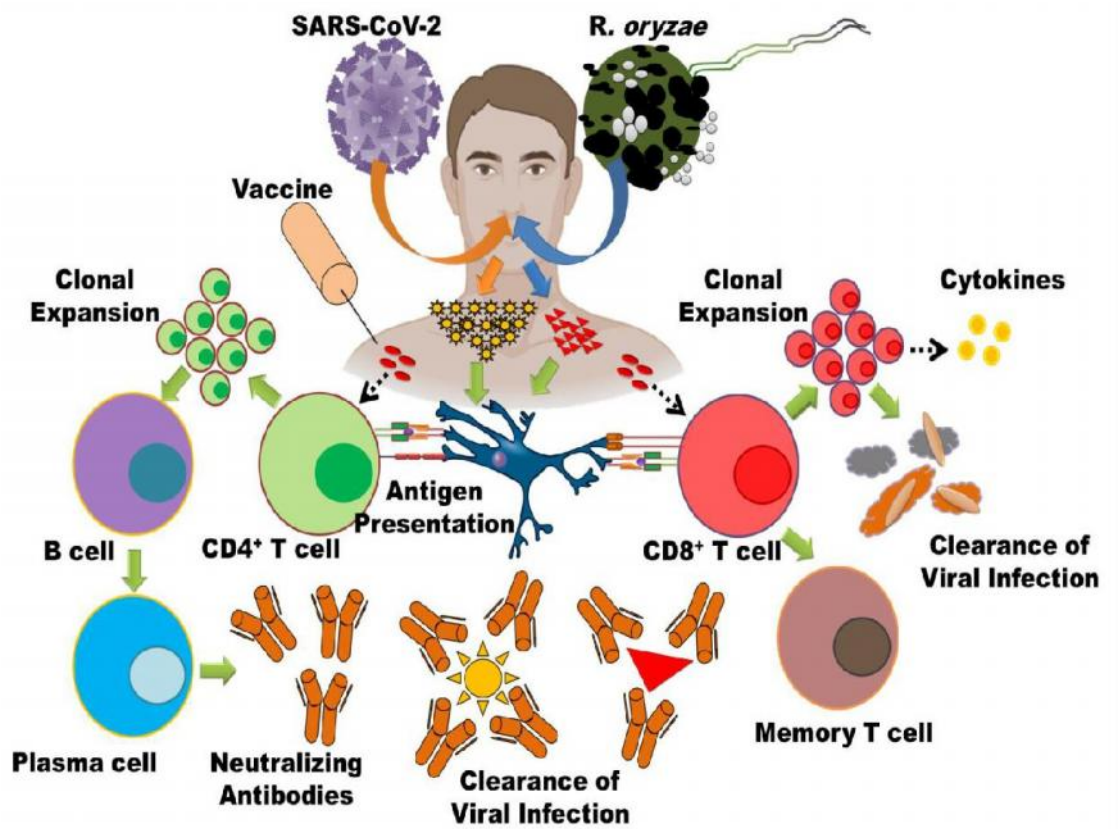


**Figure 1**. A schematic representation elucidating the role of T cells towards possible neutralization of SARS-CoV-2 and *R. oryzae* as well as a new avenue for an alternate vaccine.

## T Cell-based Vaccine: Need of the Hour

It is so possible that specific memory T cells generated against a combination of all the protein fragment epitopes of SARS-CoV-2 and Mucorales can be isolated from the infected patients. These memory T cells can be harvested and then injected into individuals as vaccine shots. In future, if the vaccinated individual acquires infection from SARS-CoV-2 and/or Mucorales, immediately the existing antigen-specific memory T cells from the vaccine in

the host will elicit a faster and more potent response toward clearing/neutralizing the virus/pathogen. In addition, the host's natural adaptive immune response will also get triggered as a result of post-infection by the pathogen, further stimulating the host cell to generate an army of T cells against the pathogen. Therefore, the immediate T cell response due to vaccination and the army T cells elicited by the hosts' adaptive immune response in post-infection will play a cohort and act orchestrated attack onto the pathogens. As a result of such coordinated, cooperative action of all the T cells will strengthen the hosts' immune system resulting in complete clearance of the virus and black fungi simultaneously.

Therefore, considering the present crisis, scientists and vaccine developers should take this opportunity of T cells as immunity booster and come forward for designing a vaccine to mitigate COVID-19 in combination with Mucormycosis. Hence, we propose designing an alternative and more effective vaccine based on the memory T cell.

A clear understanding of the role of T cells and memory T cells leads us to envision that infusing specific $CD4^+$ memory T cells in the form of vaccine shots in patients would be beneficial in mitigation of both the disease, COVID-19 and Mucormitosis, simultaneously.

## Conclusion

In summary, the infections due to the SARS-CoV-2 lead to the compromised innate immune system in host cells. Thus, the immonocomprimised COVID-19 patients are vulnerable for the secondary infection by *Micorales* species. The response of the adaptive immune systems of infected patients is the ray of hope in tackling both the disease. This is evident from a significant population of T cells generated in the host cells and their crucial role in fighting against the pathogens and recovering the patients from both the infections. The host T cells help recognise and clear the SARS-CoV-2 virus, tackle the secondary disease, Mucormicosis and balance the adverse effect of cytokines. We, therefore, envision that our study would attract the attention of the scientific community to realize the need for the development of T cell-based vaccine which would show significant efficacy for both COVID-19 and Mucormycosis, simultaneously, which is the need for the hours.

## References

1. Shah VK, Firmal P, Alam A, Ganguly D, Chattopadhyay S. Overview of immune response during SARS-CoV-2 infection: lessons from the past. Front Immunol. 2020; 11:1949.
2. To KK-W, Tsang OT-Y, Leung W-S, Tam AR, Wu T-C, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis. 2020; 20(5):565–74.
3. Tan W, Lu Y, Zhang J, Wang J, Dan Y, Tan Z, et al. Viral kinetics and antibody responses in patients with COVID-19. MedRxiv. 2020;
4. Ledford H. 'Killer' T Cells Could boost COVID immunity a mid new Variants. Nature. 2021; 590(7846):374–5
5. Stockinger B, Bourgeois C, Kassiotis G. CD4+ memory T cells: functional differentiation and homeostasis. Immunol Rev. 2006; 211(1):39–48.
6. Lees JR, Farber DL. Generation, persistence and plasticity of CD4 T- cell memories. Immunology. 2010; 130(4):463–70.
7. Fan Y-Y, Huang Z-T, Li L, Wu M-H, Yu T, Koup RA, et al. Characterization of SARS-CoV-specific memory T cells from recovered individuals 4 years after infection. Arch Virol. 2009; 154(7):1093–9.
8. Tang F, Quan Y, Xin Z-T, Wrammert J, Ma M-J, Lv H, et al. Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. J Immunol. 2011; 186(12):7264–8.

9. Tufet M. T cells calm the storm. Nat Rev Immunol. 2007;7(11):834–5. (b) Zhang B, Zhou X, Zhu C, Song Y, Feng F, Qiu Y, et al. Immune phenotyping based on the neutrophil-to-lymphocyte ratio and IgG level predicts disease severity and outcome for patients with COVID-19. Front Mol Biosci. 2020; 7: 157.
10. Ibrahim AS, Spellberg B, Walsh TJ, Kontoyiannis DP. Pathogenesis of mucormycosis. Clin Infect Dis. 2012; 54(suppl_1):S16–22.
11. Kobayashi SD, Malachowa N, DeLeo FR. Neutrophils and bacterial immune evasion. J Innate Immun. 2018; 10(5– 6):432–41.
12. Potenza L, Vallerini D, Barozzi P, Riva G, Forghieri F, Zanetti E, et al. Mucorales-specific T cells emerge in the course of invasive mucormycosis and may be used as a surrogate diagnostic marker in high-risk patients. Blood, J Am Soc Hematol. 2011; 118(20):5416–9.
13. Potenza L, Vallerini D, Barozzi P, Riva G, Gilioli A, Forghieri F, et al. Mucorales-specific T cells in patients with hematologic malignancies. PLoS One. 2016; 11(2):e0149108.
14. Schmidt S, Tramsen L, Perkhofer S, Lass-Flörl C, Röger F, Schubert R, et al. Characterization of the cellular immune responses to *Rhizopus oryzae* with potential impact on immunotherapeutic strategies in hematopoietic stem cell transplantation. J Infect Dis. 2012; 206(1):135–9.
15. Tramsen L, Schmidt S, Boenig H, Latgé J-P, Lass-Flörl C, Roeger F, et al. Clinical-scale generation of multi-specific anti-fungal T cells targeting Candida, Aspergillus and mucormycetes. Cytotherapy. 2013; 15(3):344–51.
16. Deo SS, Virassamy B, Halliday C, Clancy L, Chen S, Meyer W, et al. Stimulation with lysates of *Aspergillus terreus*, *Candida krusei* and *Rhizopus oryzae* maximizes cross-reactivity of anti-fungal T cells. Cytotherapy. 2016; 18(1):65–79.
17. Tarke A, Sidney J, Kidd CK, Dan JM, Ramirez SI, Yu ED, et al. Comprehensive analysis of T cell immunodominance and immunoprevalence of SARS-CoV-2 epitopes in COVID-19 cases. Cell Reports Med. 2021; 2(2):100204.
18. Zhao J, Zhao J, Mangalam AK, Channappanavar R, Fett C, Meyerholz DK, et al. Airway memory CD4+ T cells mediate protective immunity Against emerging respiratory coronaviruses. Immunity. 2016; 44(6):1379–91.



| **Access this Article in Online** | |
|---|---|
| | Website: www.ijarbs.com |
| Quick Response Code | Subject: Immunology |
| DOI:10.22192/ijarbs.2022.09.09.003 | |

How to cite this article:
Subhendu Sekhar Bag and Sayantan Sinha. (2022). Affinity Toward Mucormycosis in COVID-19 Patients. Int. J. Adv. Res. Biol. Sci. 9(9): 21-28.
DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.09.003