Review of: "Is SARS-CoV-2 Spike glycoprotein impairing macrophage polarization via α7-nicotinic acetylcholine receptors?"

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In this manuscript the authors aim to discuss the potential of SARS-CoV-2 Spike glycoprotein in impairing macrophage polarization via α7-nicotinic acetylcholine receptors". While the premise in intriguing this manuscript is severely flawed. It seems like that the authors focus on SARS-Cov-2 7-nicotinic acetylcholine receptors interaction with the macrophages in the first half but the completely switch gears to discuss about the unique cholinergic epitope in the virus, the failure of antibodies to neutralize mutated spike proteins as well as drawbacks of the current vaccines which in the author's opinion should be directed towards generating antibody that is capable of targeting an epitope that is conserved across all mutations.Clearly there is lack of synchronization across the 2 sections. Additionally the manuscript is rigged with vague sentences, lack of clarity and often inadequate/ missing and wrong citations.

In my opinion this manuscript is technically seriously flawed and not suitable for consideration. Please see my detailed comments below.

Detailed comments:

The innate immune cells play an important role in handling early infections, and can eliminate them completely up to a certain threshold

-handling is not a scientific word. The authors could be a bit more precise.

- 'eliminating completely' and ' certain threshold' are also vague wordings that conflict with each other. The authors should be more precise and what do they define by ' certain threshold'.

The recognition of the SARS-CoV-2 antigen triggers an eicosanoid storm and initiates a robust inflammatory response.

-what kind of inflammatory response? Innate? Adaptive? Not clear

The mechanism of this interaction, and hence the pathogenesis of the virus with the immune system, is
yet to be determined.

-Which interaction are the authors referring to? Not clear

The high expression levels of the angiotensin converting enzyme 2 (ACE2) receptor on lung epithelial cells [1][2] explains why the lung is severely affected by COVID-19,

-The explanation might be clear to the authors but not necessarily;y apparent to the readers especially if the readers are not familiar with the apparent connection between ACE2 and SARS-CoV-2 infection. The authors should make that

connection clear (spike protein binding to ACE2 receptor).

Macrophages are an attractive target due to their ability

-Attractive target for what? As a general comment, the authors need to completely rewrite/revise their writing style across the board.

They play a fundamental role in generating an adaptive immune response, as tissue-resident macrophages are poor antigen

presenting cells (APCs) and fail to migrate to regional lymph nodes [10].

-Citation 10 is more than a decade old. In a rapidly evolving field as immunology, it is pertinent to cite current literature and there is a dynamic influx of previously unknown information. Please provide appropriate and dated citations.

By keeping the macrophage population balanced towards the M1 phenotype in the microenvironment, the virus ensures an increase in the concentration of proinflammatory cytokines while avoiding the hindrance caused by anti-inflammatory cytokines **[12].**

-The citation provided (12) is not related to this statement. Citation 12 speaks about cytokine release syndrome in the context of cancer. Viral infection and particularly SARS-CoV-2 is not mentioned there, making, particularly which also makes sense since the paper was published in 2014.

At the end of the inflammatory reflex mechanism the indirect activation of α7- nicotinic Acetylcholine receptor (α7-nAChR) via Acetylcholine produced by splenic T cells inhibits splenic macrophages from expressing proinflammatory cytokines

-Is this at the end of clearing of an infection?

Whether polarization is triggered by the activation of this receptor or is only a small part of the grand polarization scheme awaits investigation, but it plays a role in the dysfunction of innate immune cells[17].

-This is a very confusing statement. In the preceding sentence, the authors state that “the indirect activation of α7- nicotinic Acetylcholine receptor (α7-nAChR) …… inhibits splenic macrophages from expressing proinflammatory cytokines”. This indicated polarization of the macrophages towards an M2phenotype. It implies to the readers that α7-nAChR regulates the polarization process and yet the authors state in this sentence that ‘it awaits investigation’. Please rephrase to clarify.

The term “error catastrophe” has not been defined for this viral candidate.

-Some readers might not be familiar with this ‘error catastrophe’ concept and needs to explained briefly for relevance.

The S2 subunit is even more highly conserved with one striking feature, the biosynthesis of a furin cleavage site that aids the transmissibility of the virus

-Citation missing

This is particularly intriguing because the cleavage of S1/S2 subunits is not even necessary for its biosynthesis

-While this is indeed an intriguing question it directly contradicts the preceding statement “The S2 subunit is even more highly conserved with one striking feature…..that aids the transmissibility of the virus”. In particular because since this feature is not needed for biosynthesis, the authors cannot comment that it is indeed the purpose of the biosynthesis of a furin cleavage site. Please rephrase.

was successful in completely neutralizing SARS-CoV but failed

to do so in this case due to the mutations observed between the S1 regions of SARS-CoV and SARS-CoV-2

-This is again a very confusing statement. The authors need to rephrase and clarify as to what do they define by “failed to

do so in this case". What is 'this case'?

This theory is supported by the rare SARS-CoV-2 antibody COVA1–16 that is also encoded to this cholinergic epitope [29].

-Which theory?

The antibody conundrum section is well written.

The issue with mRNA and adenoviral vectored vaccines is that they all encode the spike protein, which is the hidden mastermind behind a SARS-CoV-2 infection.

-It is not clear exactly where 'the issue' is. Please clarify.

Also, note that it is possible that the vaccines itself might facilitate recombinant mutations

-citation missing