Peer Review File

Article information: https://dx.doi.org/10.21037/jtd-23-846

Reviewer A

Thank you for asking me to review this paper, which is a narrative review of neutrophil function in CF and the impact of CFTR correction. Immune function in CF is a broad topic, and the authors are right to restrict themselves.

Yes. Thank you. We did restrict this review to neutrophils and cystic fibrosis. Even that is a fairly broad topic.

The paper is generally well written, though there are some instances of clumsy wording or uncorrected typos (frequent use of "intubated" rather than "incubated" for example). This is a relevant topic and the paper is a useful contribution to the literature, but would benefit from some revisions, listed below.

Yes. Thank you. We will review the manuscript carefully to correct typographical errors and poor word choices.

1. As a clinician, I found some of the details hard to follow, and the authors would do well to summarize the relevance and importance of findings at the end of each section. They do this on occasion, but inconsistently. This would help readability.

Thank you. We should do this. We will try to try to summarize each section better, especially the conclusion.

2. The authors have set out their search criteria and methods, btu have not reported on how many papers made it through each step. At the end of this they focus on a limited number of studies, which is not unreasonable but feels very selective. They have not discussed papers putting forward alternative views or not supporting the selected papers findings, or discussed important weaknesses. The overall number of quoted references is surprisingly low for a review, which again gives the impression of a selective review of the literature.

Yes. We initially used a restricted research strategy to focus on neutrophils. We will report our methods better and undertake additional searches to broaden the article recovery. We have increased the number of references.

3. The paper would benefit from some illustrations. For example a figure showing how CFTR is involved in PMN function would help to break the text up and provide a neat summary of the different papers quoted.

Thank you. We will look into this possibility and see if we have the necessary skills to provide figures to illustrate the concepts.

Minor points

HOCL in abstract should be HOCl, but also better to give it the full name. But more importantly the abstract is set out like that of a scientific paper (methods, results, conclusions), and does not fit well with a narrative review. I would suggest a more narrative abstract.

Thank you. We will revise the abstract to use a narrative format.

There is reference to sticky mucus in CF, suggesting a somewhat simplistic view of airway blockage, but no mention of the periciliary layer and the role this plays in ciliary function and hence mucociliary clearance.

Yes. This should be clarified.

Table 3 should be readable without reference to the text, but the legend and cell entries do not permit this. Needs a more complete explanation in figure legend and some relabelling to make it easier to follow. Presume these are all CF samples? In which case this needs to be clearly stated too.

Thank you. We will review table 3 and try to make it clear and understandable without reference to the text.

L173 – CFTR channel function is essential for bacterial killing – but they have just reported that this is not the case, since some killing persists in absence of functioning CFTR

Yes. This needs to be clarified.

L180 – needs reference to the clinical studies on Ivacaftor

Yes. We will add a reference to this line.

Ivacaftor is not the main treatment for CF any more, but does provide a simple shorthand for small molecule correction of CFTR function in G551D subjects, and understandably is probably the most used model of this in vitro. It would be good to include some reference to this, that CFTR correction by ivacaftor is a shorthand for CFTR correction.

Thank you.

Finally, it might be worth considering whether these results could be confounded by any direct effects of ivacaftor, which has a quinolone-like structure and therefore might (conceivably) have anti-pseudomonal activity of its own.

Yes. Thank you. We will review the literature for this possibility.

Reviewer B

Fundamentally, the search strategy introduces a bias; for strategy one, it will return only studies wherein the authors use the highly subjective term "defective", many researchers may take a more agnostic view that neutrophils are dysfunctional or simply that their function is altered. Occasionally, cell function is called its behaviour, and hence "function" may not appear at all as a term. It is a further issue that the review is titled "neutrophil function" but omits the vast array of neutrophil functions in lieu of a restricted set of functional defects. For example, the authors could make mention of neutrophil metabolism, which is skewed toward aerobic glycolysis in CF (PMID: 31454256), but because these are functions and not defects per se, they have not been covered. I would suggest re-titling the review (or broadening its scope). Indeed, from the first major paragraph to the second, there is a lurch between discussing the possibility of an intrinsic defect in the CF neutrophil and then simply depicting a facet of neutrophil function during infection. This review would be greatly aided by clarity of purpose.

The authors position the oxidative antimicrobial system of neutrophils quite centrally, but some relevant evidence is missing, such as Zhou et al. J Innate Immun. 2013;5(3):219-30., that shows CFTR is targeted to the phagosome (obviously lacking in CF).

Also, what is the justification for restricting to studies from 2000 and beyond? There is work before this this period that is of relevance. Even at the outer edge, like Coakley et al., ALP-lung, (2000).

This reviewer makes very important points regarding the limitations in the search strategy we used. We will repeat this search to look for other articles providing information relevant to the neutrophil function in patients with CF. We used the MeSH terms cystic fibrosis and neutrophils and physiology. We then then did a second search using the MeSH terms neutrophils and transmembrane conductance regulator. We changed the date restriction to all articles since 1990, thinking that the technology has significantly changed over the years and more recent articles will have better experimental detail and protocols. We also restricted articles to English language. We agree that the title of this review may be too broad since we focused on phagocytosis and microbial killing and will change the title.

The authors should update the manuscript to include studies published since February 2023, for example: Jennings et al., Neutrophil defect and lung pathogen selection in cystic fibrosis, Journal of Leukocyte Biology, Volume 113, Issue 6, June 2023, Pages 604–614. ordered

Thank you. We have reviewed this article and added information to the text.

Also, there are studies that take a more oblique look at this topic, such as Bernut, et al., Frontiers in Immunology (2020), Ng et al., Plos One, (2014) or Ortiz-Muñoz et al., JCI, (2020). The authors might consider using this kind of evidence to underscore their point about disease subsequent to dysfunctional neutrophil physiology.

Thank you. The experimental models have obvious utility investigating CF TR function. However, at present our main effort is to integrate studies involving neutrophils from CF patients and healthy controls.

Moreover, there are controversies in the nature of the CF neutrophil defect, that a reader almost expects to find re-capitulated in such a review, taking for example, McKeon et al., ERJ, (2010); Kelk et al., JCF, (2022). The authors might opt to take a side as there are certainly strengths and weaknesses of the study approaches.

Yes. We should add to that discussion to consider controversies potential weaknesses related to experimental approaches. The Kelk article reported that CF neutrophils do not have increased reactive oxygen species production after being stimulated with PMA. These authors argue that reactive oxygen species do not cause the injury and the CF lungs associated with chronic presence of neutrophils. Other mechanisms are potentially important. However, this study might suggest that the failure to respond to stimulation with increased reactive oxygen species production represents a defect that could reduce bacterial killing at the time of infection. Studies in CF patient clearly depend on the clinical status of patients and possibly other unknown confounders which influence host defense responses and inflammation. We have discussed this some in the conclusion section

On line 173 it is stated that CFTR is essential for bacterial killing, but this is untrue, in fact the evidence discussed in this review shows that, while CFTR contributes to maximal killing, that it is dispensable, with its loss causing only a moderate decrease in the rate of killing. This leads to another aspect of neutrophil physiology that ought to be touched on in the review but isn't: if CFTR dysfunction causes a defect in the CF neutrophil, is it biologically significant and clinically meaningful?

This is an important question which is potentially quite difficult to answer with any certainty. Neutrophil granules contribute to bacterial killing by adding proteolytic enzymes to phagolysosomes. Consequently, both HOCL and enzymes contribute to host defenses following infection. We need to make changes in this sentence.

Table 4: The inclusion of Sheikh et al. is an outlier here, the other studies each describe a directly tested effect of ivacaftor on neutrophils – there is a coherent link between those studies – but Sheikh et al is a study of systemic parameters that can't be attributed specifically to the neutrophil, much less to result from the action of ivacaftor on neutrophils as opposed to an indirect effect by other means. That said, there is merit to this distinct category and may warrant inclusion of similar works. For example, Casey et al., Thorax, 2023 show decline in neutrophils and neutrophil-derived proteases in the airway during ETI treatment of one year. Similarly, Schaupp et al., ERJ, 2023 show similar findings, as do Lepissier et al., AJRCCM, 2022.

Thank you. We will review these articles and try to integrate the information into a better discussion. These drugs have an important effect on inflammatory processes in the lung and systemic circulation. We can use this information to create a new table on drug effects.

Line 225 has an unclear conclusion, reflecting the unclear aim of the review. It should be made clear where neutrophil behaviours have been measured after their isolation from pwCF both pre- and post-ivacaftor versus where they have been measured in isolated neutrophils from pwCF who are treatment-naïve but whose cells are given ivacaftor ex vivo. The latter study setup is more valuable to directly address the direct effect of ivacaftor on neutrophils, though the former approach is fully valid in a discussion of the effects of ivacaftor on neutrophils – allowing that those effects may be indirect, occurring following reductions in inflammation for example.

Thank you. We will review this section and try to clarify the discussion.

Minor:-

Line 35: "This" – The- Thank you. we will make this change.

Line 35: F508del now usually preferred as Phe508del-thank you. we will make this change
Line 58: Such as- Thank you.
Line 60: what are the citations to support the claim of reduced bactericidal capacity; it's been shown but should be supported. We provided a citation for this.
Line 60: the meaning of the second sentence isn't fully clear, needs a revision. We will review this sentence. Citation 7 serves a similar purpose in the literature to the current submission. Thank you.
Line 63: elexacaftor. Thank you.
Line 64: I'm not sure it's completely accurate to say the drugs modify the protein, certainly they modulate its activity and its quantity at the membrane. We will clarify this statement.
Line 66: "3D shape" is more commonly referred to as conformation. Yes. We will make this change
Line 73: "potentiator or modulator", do the authors mean to say 'potentiator or corrector'? We will double check this word choice.
Table 1 and 2: in row 4, there is an instruction that has no place there. Assuming it is from the journal, it should probably be acted on. Thank you. We will remove this instruction
Table 2: The search strategy begins from 2014, and possibly no meaningful work testing ivacaftor on neutrophils was published prior to 2014, but nevertheless, the drug has been a published entity at least since 2010. Also, have the authors concluded that no studies report the use of a modulator other than ivacaftor? Thank you. We plan to repeat our searches to identify missing articles which are potentially relevant to this review.
Line 87: doesn't seem to make much sense; isolating primary cells for work is usually termed ex vivo work, but, once removed from the body, what other assay would one do on them? Thank you. We will double check this sentence. We will remove the words in vitro. Some clinicians may not know much about routine laboratory methods.
Line 96: the work of Painter et al (2006) has been misinterpreted here, the authors state that the P. aeruginosa were chlorinated prior to introduction to the neutrophils, but this is incorrect, as the entire point of the experiment was to assess the ability of the (CF) neutrophil to do so. Thank you. We will review this sentence and this work. This sentence was changed.
Line 97: "spectrophotometry" should be spectrometry. Thank you
Line 104: the conclusion drawn from the Painter study is broadly correct, but could do with strengthening; this and other studies show that CFTR is absent from the phagosomal membrane, hence its function there is lacking. It is also a consideration as to which classes of variants show this consequence and to what extent. Okay. Thank you. We will review this section and try to make it clearer. The Zhou article demonstrates that the CRTR protein moves to the phagosome membrane. It is possible that the protein in patients with other less common mutations do not do that. However, that information is not available.
Line 113: should say Stimulation Index-yes
Line 116: should say stimulated- Yes
Line 128: bacteria with neutrophils- Yes
Line 133: suggest re-phrasing for clarity-we will double check this sentence
Line 138: capitalise NET-Yes, we will make this change.
Line 146, 151: should say incubated-yes. Thank you
Line 148: speculates as to harm pursuant to excess calcium in the neutrophil, but this contention should be supported. Presumably it stems from the finding of lower NET release, but this could be clarified. We will try to clarify this statement.
Table 4, row 2: the reference should be 2016. Yes. Thank you
Line 182: Haynes should be Hayes- Thank you
Line 193: a gating mutation- Yes, we will make this change.

Line 205: Levels of mRNA transcript and activity of the translated protein don't always correlate; here, they happen to so we can infer that de novo synthesis of HV1 is contributing to enhanced ROS production. Yes. Thank you

Line 207: The overall findings of Guerra et al., are undersold; it is part of a body of literature that have studied ivacaftor effects in vivo, longitudinally, and found its effectiveness waning over time. To date, triple-therapy has yet to show such a reversion (eg in Casey et al, Sheikh et al, Schaupp et al, etc), with the possible exception of Pallenberg et al., Micro Spectrum, (2022). Yes. We will review these articles and make changes in the discussion

Line 212: F508 should be Phe508del-We will make this change.

Line 213: form should be from. We will make this change.

Reviewer C

This manuscript provides an overview of neutrophil dysfunction in CF with a focus on reactive oxygen species production and the effect of the modulator ivacaftor on CF neutrophils.

While the authors convincingly justify the importance of neutrophils in CF, the review focuses mostly on the production of reactive oxygen species by CF neutrophils. Other neutrophil functions are also dysregulated in CF such as NET formation. NETs play a key role in CF. This is partly explained by the literature search methodology that used a limited choice of search terms. Also, the title gives the impression that this is a review on neutrophil functions in general and not focused on reactive oxygen species. A more extensive search of the literature is required to capture all the key findings regarding neutrophil dysfunction in CF.
Yes. We agree. We will repeat our search to identify additional articles that help explain other neutrophil functions in CF patients.

Additional details that require improvement include:

1. making it clear that there are several hundred CF mutations yes. We will not add that information.

2. studies are explained in too much detail leaving less space for the discussion of the findings,
We will review this study detail and see if it can be shortened. We have added to the discussion.

3. avoid general statements as the one on line 225, this phrase is too general, I suggest listing the functions identified as being modulated by ivacaftor. We will review this line and try to avoid generalizations
4. other modulators should be mentioned as well. Yes. Thank you.

5. the word 'intubated' should be replaced with 'incubated'. Yes. We will make this change.