Peer Review File
Article information: https://dx.doi.org/10.21037/aob-22-40

Reviewer Comments

The paper titled “Atypical Hemolytic Uremic Syndrome: Genetically-based insights into pathogenesis through an analysis of the complement regulator CD46” is interesting. This review focus on insights derived from the assessment of rare variants in CD46. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: How alternative pathway activation proceeds and how defective control increases activation, which ultimately leads to endothelial cell damage? It is suggested to add relevant contents.

Reply 1: We have now devoted several sections (Pages 5-8) to further describe complement activation and its regulation in order to better introduce the complement system. In particular, we added more on basics of the alternative pathway and its role in disease pathogenesis on pages 6 and 17.

Comment 2: Is the fundamental defect of aHUS an excessive complement attack against the cell surfaces? What are the possible reasons? It is suggested to add relevant contents.

Reply 2: We do believe it is a lack of adequate membrane regulation that drives the pathogenesis of aHUS. To emphasize this point, we wrote a new section on page 17: "Pathogenesis of aHUS. Reduced control of complement activation (specifically cofactor activity) on the cell membrane is the essential pathogenetic mechanism of aHUS. Mutations of membrane regulator CD46 or serum protease CFI contributed to inadequate regulation of complement activation on the cell membrane (24). Also, many of the CFH mutations associated with aHUS occur in its C-terminal region (CCPs 16-20) where CFH binds to a cell membrane through GAG. The phenotype of CFH C-terminal null mutant mice was similar to that of the aHUS while the cross of this mutant mouse into a C5 deficient background led to suppression of disease production (24). Finally, most autoantibodies against CFH in aHUS patients predominantly recognize the C-terminus of CFH. These observations indicate that the effective removal of activated fragments of complement on endothelial cells plays a key role in the pathogenesis of aHUS."

Comment 3: How advances in the understanding of aHUS pathogenesis have impacted on prevention and cure of aHUS recurrence after kidney transplantation? It is suggested to add relevant contents.

Reply 3: Relative to transplantation development and management, we added a paragraph on page 18: "Patients with aHUS who progress to end stage kidney disease are candidates for transplantation. Renal transplantation had been contraindicated because of the high risk of disease recurrence leading to early allograft loss especially

in patients with heterozygous rare variants in CFH or CFI. However, over the past decade, advances in our understanding of the pathophysiology of aHUS and availability of highly efficacious anticomplement drugs have permitted successful kidney transplantation in these patients. Therefore, all patients with aHUS should undergo an evaluation to screen for complement system abnormalities"

Comment 4: The “Introduction” part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Atypical hemolytic uremic syndrome after childbirth: a case report, PMID: 33553372”, “Gemcitabine-induced thrombotic microangiopathy treated with eculizumab: a case report, PMID: 36636054 ”. It is recommended to quote the articles.
Reply 4: We appreciate your comments and suggestions. As noted above, we specifically added a paragraph to further outline the alternative pathway on page 6: "The activation of complement classical (CP) and lection (LP) pathways are mediated mainly by antibody and lectins, respectively. The latter interact with carbohydrate components of microbes. The alternative pathway (AP) may be activated by spontaneous hydrolysis (tickover/turnover) of C3's thioester bond to form C3 (H2O). It is also a powerful amplification loop that enhances the initial activation by CP and/or LP. Employing C5 activation as a measurement for total complement activation in several well studied examples, the AP contributed up to 80% of its generation (7). This point further illustrates the importance of tight regulation of AP."

We thank you for providing the two papers. They are interesting but we felt they were relatively unrelated to the topic of our review.

Comment 5: It is suggested to increase the research progress of pathogenesis of aHUS in the discussion.
Reply 5: Regarding the pathogenesis, we added a paragraph (see our answers to your question number 2). In addition, we wrote three paragraphs (pages 9-10) on an in-depth prior review and the progress largely since this article was published (19).

Comment 6: What are the clinical characteristics of aHUS associated with abnormal complement regulation? It is suggested to add relevant contents.
Reply 6: The clinical characteristics of aHUS are due to poorly/inadequately uncontrolled complement activation contributing to the damage of endothelial cells and the generation of thrombosis. We have chosen to focus on the most frequently involved organ, the kidney, and renal transplantation. Our review has covered basic mechanisms and key clinical manifestations that lead to aHUS if you have a rare dysfunctional variant in CD46. Other than a milder overall clinical course and a more favorable response to transplantation, we are not aware of any major clinical manifestations in aHUS secondary to CD46 vs. CFH or CFI rare variants.