**BRAIN-REVIEW**

The manuscript entitled "TLR4 mediates inflammatory injury via MyD88/TRIF signaling pathway in intracerebral hemorrhage" by Sen Lyn et al, explores the role of TLR4 in ICH-induced inflammation. In order to elucidate this question, in the first, step the authors analyzed TLR4 mRNA expression at different times after ICH. The results demonstrated that TLR4 mRNA is up-regulated in perihematomal brain regions. The authors next assessed cellular expression of TLR4 and found that TLR4 is up-regulated in neurons, astrocytes and CD11b+ cells of ICH animals. Next, Sen Lyn and coworkers induced ICH in TLR4 -/- mice and found less edema and a lower neurological deficit in these animals when compared to wild types. In the same animals (TLR4 -/-), the authors also observed a lower amount of proinflammatory cytokines and macrophage infiltration. The authors explored which signal pathway is involved in TLR4-inflammatory response after ICH. They found that both MyD88 and TRIF were implicated. In the same way, it was shown that MyD88 and TRIF participate in TLR4 mediated inflammation in response to ICH via activation of NF-kB. After a set of *in vitro* experiments, Sen Lyn and coworkers showed that heme and $Fe^{2+}$ are capable of potentiating microglial activation and inducing a TLR4-mediated inflammatory injury. Finally, the authors demonstrate that monoclonal antibodies against TLR4 promote neuroprotection following ICH.

The authors' final conclusion was that heme and $Fe^{2+}$ trigger a TLR4-mediated inflammatory injury via the MyD88/TRIF signaling pathway in ICH. This conclusion only involves the last part of the experiments performed! The only novel contribution, the participation of MyD88/TRIF signaling in ICH, has already been reported.

It is worthwhile to mention that despite the work's large set of well designed and executed experiments, my concern about the novelty of the manuscript was always latent. A large part of the work examines the role of diverse factors that have already been explored in other models of cerebral diseases and even in experimental models performed under the same context. For instance, the role of TLR4 after subarachnoid hemorrhage has already been described by Zhou and coworkers (Brain Res. 2007, 1173:110-6). In this case, even though the animal model is different (subarachnoid vs. intracerebral hemorrhage), the final conclusion regarding the role of TLR4 in inflammation is the same. What moved the authors to think that the role could be different? This should be clarified in order to understand the new contributions. Other findings of the work have already been described. For example, it has been reported that blood components like oxihemoglobin can induce the expression of TLR4 (Brain Res. 2010;1322:102-8). Moreover, in the present work, Sen Lyn and coworkers discussed that TLR4 has already been identified as being capable of binding with heme and inducing TNFα secretion. Therefore, Sen Lyn is reporting the same phenomenon but in a different model. Another concern arises from the finding related to $Fe^{2+}$. With this regard, the authors discuss that, in a previous study, only heme but not $Fe^{2+}$ could activate macrophages via TLR4 to produce TNFα. To explain the discrepancy, the authors claim that it could be due to the different cell type used for the experiment; however, they do not provide any logical or convincing argument as to underline this as a possible cause of the discrepancy. This topic should be revised.

Aside from the above observations, there are some major and minor issues that must be clarified or carried out before it can be considered for publication.

Major issues:

1. The CD11b marker was used to identify microglia in tissue samples. With this regard, the authors should be aware that CD11b is not a specific marker for microglia; it is also expressed by peripheral macrophages, granulocytes, natural killer cells and CD8+ lymphocytes. Therefore, several of the labeled cells could have not been microglia.

2. An interesting approach was the administration of anti-TLR4 antibodies (already reported in ischemia-reperfusion models). This approach allows for a therapeutic translation of the laboratory into the clinical setting; however, the antibodies were administered 3 days before the ICH. This makes it difficult to evaluate the usefulness of this therapy from a clinical standpoint. Why didn't the authors administer the antibodies immediately after blood

infusion? or, why not administer the antibodies at different times after infusion? This could provide more relevant information for potential clinical studies.

Minor issues:

1. The entire manuscript presents language problems, there are typographical, spelling and grammar errors, which make the meaning of many sentences difficult to understand. For instance:
    1. Page 8: A blunt 26-“guage”
    2. Page 13: IL-β
    3. Page 21: intracetoplasmic
    4. Page 24: Above data showed that TLR4 was significantly upregulation in …
    5. Page 30: accumulation of heme in perhematorma….

COMMENTS TO THE EDITOR

The manuscript is well executed; however, the real novelty is not clear, almost all the findings have already been described in the same or other models of neurodegenerative diseases. Considering that original papers published in Brain represent more than just an increment in knowledge and are likely to be definitive articles of lasting value in their field I do not recommend the publication of this manuscript.

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Authors: Yang, qingwu (contact); Lin, Sen; Zhong, Qi; Lv, Fenglin; Zhou, Yu; Li, Junqi; Wang, Jingzhou

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## ASSESSMENT

**Scientific Interest**

- ☐ Outstanding
- ☐ High
- ☑ Medium
- ☐ Low

**Clinical Interest**

- ☐ Outstanding
- ☐ High
- ☑ Medium
- ☐ Low

**Originality**

- ☐ High
- ☑ Medium
- ☐ Low

**Length**

- ☐ Too Long
- ☑ Appropriate
- ☐ Too Short

**Tables**

- ☐ Too Many
- ☑ Appropriate
- ☐ Inadequate

**Figures**

- ☐ Too Many
- ☑ Appropriate
- ☐ Inadequate