March 12, 2013

Dear Editor-in-Chief

We want you to consider in detail the paper published in the January 2013 issue of *Journal of Clinical Biochemistry and Nutrition*, by Tomoki Aomatsu et al. entitled “Neutralization of complement component C5 ameliorates the development of dextran sulfate sodium (DSS)-colitis in mice.” The authors have evaluated the pathophysiological role of the complement system in a mice colitis model. To study the role of complement, they have used Eculizumab, an antibody directed towards the human complement protein C5. We have two strong issues against this study, both regarding the use of the C5-neutralizing antibody, Eculizumab.

1. The Eculizumab, whose CDRs are of mouse origin, is highly specific against human C5, and has shown not to bind C5 of any other tested species. Aomatsu et al. will not neutralize C5 in the mouse when giving Eculizumab, and the effect they report from the Eculizumab is thus complement independent. To verify that Eculizumab indeed not cross-react with mouse, and that our argue is valid, we tested the complement inhibitory effect of Eculizumab in mouse serum in vitro in our laboratory, and found no effect on mouse complement activity as evaluated in CH50 using adequate controls (Fig. 1).

2. A C5-neutralizing antibody must be given in a dose at least 100 times higher than what was used by Aomatsu et al. C5 circulates in mouse plasma (male) at a level of approximately 90 µg/mL (= 0.5 µM). Eculizumab was given in a dose of 1 µg/body every 48 h, which corresponds to a serum concentration of approximately 5 nM. The standard antibody for neutralizing mouse C5 is the IgG1 BB5.1. The supplier recommends a dose of 40 mg mAb/kg (approx 1 mg/body) 0–2 days followed by injection twice a week. To note, however, even if they had used a sufficient dose, it would have had no effect, due to the lack of specificity as mention under item 1.

We are surprised that this paper passed referees desk without noting this. We have sent two separate e-mails to the authors and asked for documentation that Eculizumab worked in their mice, but we have got no answer.

To summarize, the effect that Aomatsu et al. report in the intervention group is not an effect of neutralization of C5. The complement system is involved in several inflammatory disorders, likely also in colitis. The nature of complement involvement in colitis has to be carefully evaluated and not to be misled by this study. Therefore, we strongly suggest you to consider retraction of this paper.

Looking forward to hear your reply.

Sincerely,

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March 15, 2013

Dear Editor-in-Chief,

I read a letter from Professor Tom Eirik Mollnes, Oslo University. They raised some issues about our results appeared in J Clin Biochem Nutr 2013; 52: 72. We presented the data that the anti-C5-neutralizing antibodies, Eculizumab, prevented the development of dextran-sulfate sodium (DSS)-colitis in mice. We repeatedly confirmed in vivo effects of Eculizumab by the several markers such as body weight loss, the disease activity index, the colonic weight/length ratio, histological colitis score, mucosal myeloperoxidase activity and mucosal cytokine mRNA expression. We have no doubts of in vivo effects of this drug in our model. However, we have no data about inhibition of complement activity in our model.

The issues raised by Prof. Mollnes et al. are based on their in vitro data that Eculizumab is specific for human but does not work in mice. Their data shows Eculizumab does not inhibit mouse complement-mediated lysis in vitro. So, they thought that our in vivo results in mice are independent on inhibition of complement activation.

Since we have no data of complement activities in our model, we cannot make a complete answer. We can simply say that this drug inhibited DSS-colitis in mice. We deeply appreciate the comments by Prof. Mollnes, and our results may include the possible mechanisms independent on complement activation. We will pay attention to the issues raised by Prof. Mollnes in the future experiments in murine models. In addition, further experiments to explore the mechanisms underlying in vivo effects of this drug, including non-complement inhibition, will be performed.

Sincerely yours,

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