To the editor:

Eculizumab treatment efficiently prevents C5 cleavage without C5a generation in vivo

We read with interest the letter by Burwick et al in Blood and were surprised by their conclusion that eculizumab failed to inhibit C5a generation in vivo. Eculizumab is a monoclonal antibody binding to human C5 preventing its cleavage to C5a and C5b. The authors investigated plasma concentrations of C5a and sC5b-9 in 1 patient with hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome treated with eculizumab.

They showed that, before the start of eculizumab, C5a concentration was low, whereas the sC5b-9 concentration was substantially elevated. This is consistent with what frequently is found when the terminal pathway is activated in vivo: sC5b-9 has a long half-life of ~60 minutes compared with the very short half-life of ~1 minute for C5a because of binding to the leukocyte C5a receptors. Thus, it is a typical pattern of human in vivo complement activation that is described for the HELLP patient at baseline. Immediately after the start of eculizumab, the sC5b-9 concentration fell abruptly, consistent with inhibiting C5 cleavage. Surprisingly, the C5a concentration apparently started to increase when eculizumab was initiated. Because this observation was unexpected and did not fit with any previous data, we aimed to reproduce these data and search for possible explanations for the findings.

Thus, we examined 3 patients with atypical hemolytic uremic syndrome (aHUS) that started with eculizumab and followed them over time for plasma C5a and sC5b-9. Because we suspected that the C5a enzyme-linked immunosorbent assay (ELISA) kit the authors had used (BD Bioscience, San Jose, CA) could have given false-positive results, we included this kit (BD HU C5a OPTEIA Kit II, Cat. No. 557965), as well as 2 other well-established C5a ELISA kits in our study (RND Duoset Human Complement Component C5a, Cat. No. DY2036; RND Systems, Minneapolis, MN; and Hycult C5a, Human ELISA kit, Cat. No. HK394-02; Hycult Biotech, Uden, The Netherlands).

Notably, the BD C5a assay detected an abrupt increase in C5a in all 3 patients immediately after eculizumab treatment was started, whereas no increase was found using the RND or the Hycult (HC) kits (Figure 1A). Plasma sC5b-9 immediately decreased in the aHUS patients, as described for the patient with HELLP syndrome, using the same assay as Burwick et al, consistent with efficient blocking of C5. We then tested the effects of eculizumab in vitro by activating human serum and convincingly documented that eculizumab efficiently blocked C5a as measured by both the BD and the RND kits, as well as sC5b-9 measured by a singleplex assay developed in our laboratory (Figure 1B).

C5 might be cleaved directly, without a traditional C5 convertase (ie, in the absence of C3). However, it has never been shown that C5a is released without simultaneous C5b-9 formation. In fact, Krisinger et al showed that an even more effective C5b-9 complex was formed by direct cleavage of C5 by thrombin. Furthermore, C5-9 formation has been documented by a conformational change of C5, making a “C5b-like” molecule generating a C5-9 complex without release of C5a. Generation of C5a, without formation of C5b-9, has never been documented, and the data by Burwick et al do not document this, but rather reveal a false-positive reaction in their assay.

The authors described a patient with the HELLP syndrome. One possible explanation for their findings would be that this patient was exposed to an antigen related to this particular disease, detected in the BD C5a assay. This was definitely not the case, because the 3 patients we describe had aHUS and showed the same pattern. Thus, this reactivity seems to be directly related to eculizumab treatment. Interestingly, we found a correlation between the BD C5a assay and the eculizumab-C5 complexes, in an assay described by us recently.

These data underscore the importance of confirming unexpected and surprising data by using alternative and different assays, instead of relying on a single assay. The authors are to be commended for their efforts to study complement activation with eculizumab treatment, and the unexpected results of our study are not unexpected anymore, because we could reproduce the phenomenon in vivo.

![Figure 1. Effect of eculizumab on C5a and sC5b-9 generation in vivo and in vitro](image-url)
Response

Maternal and cord C5a in response to eculizumab

We appreciate the interest of Volokhina et al in our recent letter to the editor in Blood. Their data on measurement of C5a in human plasma in response to eculizumab adds to the scant literature on this topic. They raise 2 criticisms that we did not address in our original letter: (1) plasma C5a levels may be spuriously elevated due to cross-reactivity with other epitopes specific to the BD C5a enzyme-linked immunosorbent assay (ELISA) (BD Bioscience, San Jose, CA), and (2) eculizumab-C5 (E-C5) complexes may be the source of cross-reactivity. These are both important considerations.

Although the data were not included in our initial report, we also measured umbilical cord plasma levels of C5a in our patient with preeclampsia and hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome using the BD C5a ELISA. Umbilical cord plasma C5a levels were measured at 94.1 ng/mL (56% of maternal plasma levels). The umbilical cord plasma levels of C5a in 10 randomly selected severe preeclampsia cases not on eculizumab measured 19.3 ± 8.2 ng/mL (median, 58.8% of maternal levels). Similar cord C5a measurements were noted in 3 HELLP cases not on eculizumab (26.7 ± 9.5 ng/mL; median, 61.0% of maternal levels). In light of the findings of Volokhina et al, the higher levels of cord C5a detected in our preeclampsia/HELLP patient treated with eculizumab may reflect increased levels of E-C5 complex. The detection of E-C5 complex is an important consideration, because E-C5 complexes are capable of crossing the placenta and may also be deposited in the kidney. Hallstensen et al estimate that newborns carry 6% to 7% of the E-C5 complex detected in their eculizumab-treated mothers with paroxysmal nocturnal hemoglobinuria. Nonetheless, it is also noted that the ratio of umbilical cord C5a to maternal C5a was similar between our preeclampsia/HELLP patient treated with eculizumab and our preeclampsia/HELLP cases not exposed to eculizumab. In addition, as we previously reported, baseline (pretreated) plasma C5a levels (measured by the BD C5a ELISA) in our case study were high compared with healthy pregnant controls and subjects with severe preeclampsia. Together, these data suggest that E-C5 complexes may not be the sole factor contributing to elevated maternal and cord C5a readings in the BD assay.

Although we have successfully used eculizumab to treat severe preeclampsia/HELLP syndrome and believe that it is a promising treatment of this condition, we feel that plasma C5a levels may be less helpful than other markers of complement activation. C5 products that support the terminal complement activation pathway.

References


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