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BASIC SCIENCE ARTICLE

Dual inhibition of complement C5 and CD14 attenuates inflammation in a cord blood model

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BACKGROUND: *Escherichia coli* and Group B streptococci (GBS) are the main causes of neonatal early-onset sepsis (EOS). Despite antibiotic therapy, EOS is associated with high morbidity and mortality. Dual inhibition of complement C5 and the Toll-like receptor co-factor CD14 has in animal studies been a promising novel therapy for sepsis.
METHODS: Whole blood was collected from the umbilical cord after caesarean section ($n = 30$). Blood was anti-coagulated with lepirudin. C5 inhibitor (eculizumab) and anti-CD14 was added 8 min prior to, or 15 and 30 min after adding *E. coli* or GBS. Total bacterial incubation time was 120 min ($n = 16$) and 240 min ($n = 14$). Cytokines and the terminal complement complex (TCC) were measured using multiplex technology and ELISA.
RESULTS: Dual inhibition significantly attenuated TCC formation by 25-79% when adding inhibitors with up to 30 min delay in both *E. coli*- and GBS-induced inflammation. TNF, IL-6 and IL-8 plasma concentration were significantly reduced by 28–87% in *E. coli*-induced inflammation when adding inhibitors with up to 30 min delay. The dual inhibition did not significantly reduce TNF, IL-6 and IL-8 plasma concentration in GBS-induced inflammation.
CONCLUSION: Dual inhibition of C5 and CD14 holds promise as a potential future treatment for severe neonatal EOS.

Pediatric Research; https://doi.org/10.1038/s41390-023-02489-2

IMPACT:

- Neonatal sepsis can cause severe host inflammation with high morbidity and mortality, but there are still no effective adjunctive immunologic interventions available.
- Adding CD14 and complement C5 inhibitors up to 30 min after incubation of *E. coli* or Group B streptococci in a human umbilical cord blood model significantly reduced complement activation and cytokine release.
- Dual inhibition of C5 and CD14 is a potential future therapy to modulate systemic inflammation in severe cases of neonatal sepsis.

INTRODUCTION

Despite advances in neonatal medicine, early-onset sepsis (EOS) still remains a significant cause of morbidity and mortality due to cases with severe host inflammation and limited protective responses.[1,2] EOS is caused by vertical transmission of bacteria colonising the gut and the birth canal. Together, *E. coli* and Group B streptococci (GBS) cause approximately 2/3 of all EOS cases. Both bacteria may cause severe disease with high risk of later sequela or death, in both term and in particular preterm infants.[3,4]

The complement system and the cytokine network are key players of the innate immune system, centrally involved in the host inflammatory response.[5] The complement system is activated by three routes; the classical pathway, the lectin pathway and the alternative pathway (Fig. 1).[6,7] These pathways merge at C3, which when activated, binds to bacteria. This opsonisation leads to enhanced phagocytosis and is the main mechanism of the complement system in bacterial defence. Other complement mediated mechanisms are lysis of some Gram-negative bacteria by the membrane form of the terminal C5b-9 complex and through C5a-enhanced synthesis of inflammatory mediators and degranulation of granulocytes.[8] Both *E. coli* and GBS activate the complement system,[7–11] but in severe cases this activation can be excessive and lead to harmful inflammation.[12] During sepsis, cytokine concentrations increase exponentially through a multitude of different pathways, including activation of pattern recognition receptors, e.g. Toll-like receptors (TLRs).[9–11] Under normal conditions, TLR-activations induce a local and self-limited response. However, in sepsis TLR-activation can be improper and uncontrolled, leading to a fatal systemic imbalance.[12]

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Received: 8 February 2022 Revised: 20 December 2022 Accepted: 13 January 2023
Published online: 01 February 2023

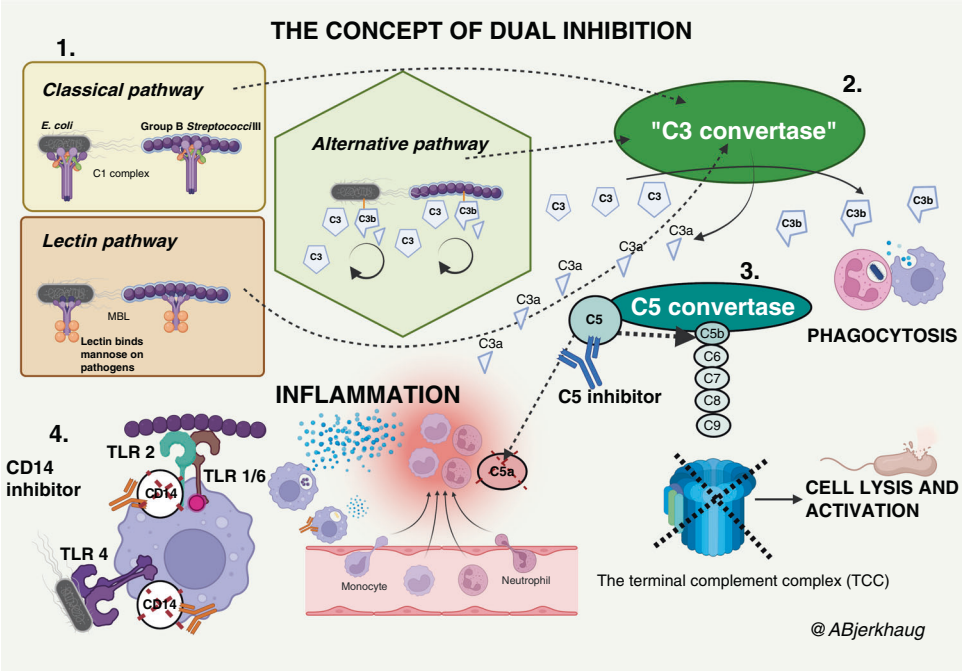

Fig. 1 Graphical abstract: cartoon of the dual inhibition of complement C5 and the TLR CD14 co-receptor. (**1**) *E. coli* and GBS entering the bloodstream are rapidly opsonized by complement components and immunoglobulins. (**2**) C3 convertases cleave the central and the most abundant component of the complement system, complement C3, that gives rise to the inflammatory fragment C3a and opsonisation fragment C3b. (**3**) The membrane form of the terminal C5b-9 complex induces lysis of bacteria and in sub-lytic amounts activates cells, while the role of C5a includes enhanced synthesis of inflammatory mediators and degranulation of granulocytes. **(4)** Dual inhibition targeting complement C5 and CD14 reduces inflammatory response without interfering with the C3 opsonisation.

A range of adjunctive inflammatory interventions have failed to show convincing beneficial effects in treatment of neonatal sepsis.[13] Complement factor C5 and the TLR co-receptor CD14 are new potential targets for sepsis therapy.[12] C5 inhibition reduces the proinflammatory effects caused by C5a and the terminal C5b-9 complex (Fig. 1). CD14 is a co-receptor for several TLRs,[14] including TLR2 and TLR4, expressed on macrophages and neutrophils (Fig. 1). CD14 plays an important role in the detection of lipopolysaccharides (LPS) from Gram-negative bacteria, but also other pathogen-associated molecular patterns such as lipoteichoic acids (LTA) from Gram-positive bacteria.[12,14–16] Studies in Gram-negative ex vivo and animal models have indicated that the dual inhibition of C5 and CD14 may be beneficial in attenuating the detrimental effects of complement activation, and to modulate the cytokine storm in fulminant sepsis.[12] Similar beneficial effects have been observed in experimental models with *Staphylococcus aureus*.[17] However, in most of these studies the dual inhibition of C5 and CD14 has been "prophylactically" administered, as proof of concept, before induction of sepsis.[12]

In neonates with sepsis, empiric antibiotic therapy must cover the most commonly seen pathogens, and therapy is often started after the baby has become symptomatic.[18,19] A similar approach would be necessary for empiric immunomodulatory treatment. The main objective of this study was to compare *E. coli*- and GBS-induced inflammation and to evaluate the effects of dual C5-CD14 inhibition in an ex vivo human umbilical cord blood model.

METHODS

Study groups and blood collection

Mothers scheduled for an elective caesarean section at the University Hospital of North Norway in the period of October 2019 and September 2021 were invited to participate. In sub-study 1, we collected cord blood samples ($n = 16$) and incubated blood with bacteria for 120 min before analyses (Fig. 2). After an interim analysis of data, we found that the cytokine release after 120 min, especially after GBS incubation, was lower than expected from previous studies with other bacteria (*E. coli* and *S. aureus*).[17,20–23] We then performed new pilot studies in both adult and cord blood (Supplementary Fig. 1a–h). Subsequently we decided to perform sub-study 2 where we collected cord blood samples ($n = 14$) and now incubated blood with bacteria for 240 min before analyses.

Bacterial strains and culture conditions

E. coli strain LE392 (ATCC 33572; Manassas, VA) and a clinical GBS strain, serotype III (SO-SAG18-1, kindly provided by the Norwegian GBS reference laboratory, Trondheim, Norway) were used in all experiments. We slightly adapted a previously described heat inactivation protocol[20] for both bacteria. *E. coli* was grown in Luria-Bertani (LB)-medium and GBS in Todd Hewitt medium overnight, then washed once (3200 × *g*, 10 min at 4 °C) with 50 mL phosphate-buffered saline (PBS; Sigma-Aldrich, Steinheim, Germany). After resuspension in PBS, *E. coli* and GBS were separately heat-inactivated for 1 h at 60 °C. Growth controls after heat-inactivation confirmed that all bacteria were killed. *E. coli* and GBS were batched and frozen at −70 °C.

Upon use, the heat-inactivated strains (*E. coli* and GBS) were thawed and washed six times in PBS, as described above. Absolute bacterial counts were obtained by diluting the bacteria 1:500 with PBS, followed by transfer of 2450 µL to a tube designed for compatibility with the flow cytometer. We added 50 µL CountBright® (Life Technologies Corporation, OR). The samples were run on a flow cytometer (BD Biosciences, NJ) and the concentration of *E. coli* and GBS were calculated using the formula provided in the CountBright® instruction manual. A batch suspension of 7.17×10^7 *E. coli*/mL PBS and 6.17×10^7 GBS/mL PBS was made and kept at 4 °C for up to maximum four months.

Inhibitors

The complement C5 inhibitor, eculizumab (Soliris®) was obtained from Alexion Pharmaceuticals (Boston, Ma). The recombinant anti-human CD14 IgG2/4 antibody (r18D11) and an isotype-matched control were produced in our laboratory, as previously described.[24]

Ex vivo human cord blood model

Both the original ex vivo whole-blood model[25] and the post-challenge model[22] have been described in detail previously. The major advantage of

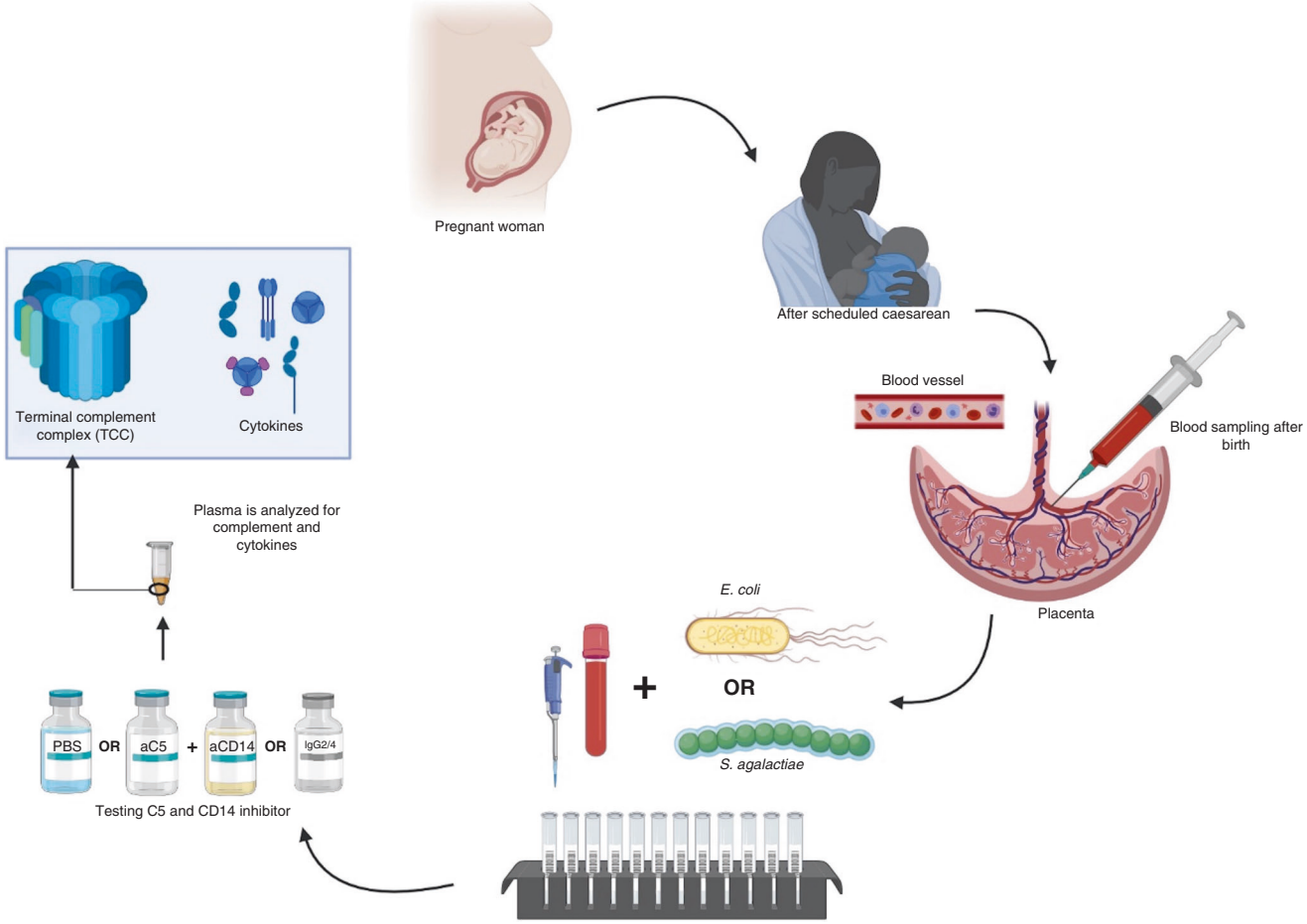

Fig. 2 Graphical abstract: cartoon of the experimental set-up of the study.

the ex vivo human whole-blood model is the use of the thrombin-specific inhibitor lepirudin, which does not interfere with the complement system or the inflammatory network, in contrast to other frequently used anticoagulants such as EDTA, citrate and heparin.[24]

A time course for our study is shown in Supplementary Fig. 2. In the current study, we aimed to optimise cord blood sampling volumes, but volumes obtained were usually just enough for all the planned analyses including controls and inhibition experiments. All cord blood was drawn into endotoxin-free 4.5 mL NUNC tubes (Thermo Fischer Scientific, Roskilde, Denmark) and lepirudin (Refludan®, Pharmion, Windsor, UK) was added to a concentration of 50 µg/mL blood. Several pilot experiments in adult blood were performed before the main study in order to assess the effect of single versus dual inhibition and to compare bacterial challenge with 10^7 GBS bacteria/mL versus 10^8 bacteria/mL (Supplementary Fig. 3a–h).

In sub-study 1, the baseline sample (T0) was processed less than 20 min after the blood was drawn. Combined inhibitors eculizumab (final concentration 100 µg/mL blood) and anti-CD14 (final concentration 15 µg/mL blood) or isotype-matched control IgG2/4 (final concentration 15 µg/mL blood), were added to separate tubes at each of the following time points: 8 min prior to, and 15 and 30 min after adding *E. coli* or GBS to a final concentration of 10^7 bacteria/mL whole blood.[22,25] Two positive controls were incubated with either *E. coli* or GBS. The negative control was incubated with PBS only. All samples were incubated in a Rotamix Intelli-Mixer (Norengros, Oslo, Norway) with rotation of blood at 37 °C for 120 min after adding bacteria or PBS. Complement activation was stopped by placing the samples on ice and adding EDTA (Sigma-Aldrich, Steinheim, Germany) to a final concentration of 20 mM. The samples were centrifuged for 20 min at 3000 × *g* at 4 °C. Plasma was collected and stored at −70 °C until analysed. Sub-study 2 followed the same protocol, but the samples were incubated for 240 min instead of 120 min.

Cytokine multiplex assay

Pro-inflammatory cytokines (TNF, IL-6 and IL-8) were measured using a multiplex cytokine assay (Bio-Rad Laboratories, Hercules, CA). The assay was performed according to the manufacturer's instruction.

Enzyme immunoassays for complement activation products

The soluble terminal C5b-9 complement complex (TCC) assay was performed according to a method developed in our laboratory and described in detail previously.[26] In short, the principle of the TCC assay is based on a monoclonal antibody aE11 reacting with a neoepitope expressed in C9 only after it is activated and incorporated into the C5b-9 complex. TCC concentrations are reported as complement activation units (CAU)/mL.[26]

Data presentation and statistics

GraphPad Prism version 9.2.0 (GraphPad, San Diego, CA) was used for statistical analysis and presentation. Descriptive results are presented as means with standard deviation (SD) and medians with range or interquartile range (IQR; 25–75 percentiles). When comparing the effect of dual inhibition of *E. coli* or GBS induced inflammation at different time points with the positive control group, the results were analysed by the non-parametric Wilcoxon matched-paired signed-rank test. Percentage inhibition of the positive control is presented related to the negative control as baseline. A p value <0.05 was considered statistically significant for all analyses.

RESULTS

Thirty mothers were included in this study. Two had mild preeclampsia, 1 had a well-controlled diabetes mellitus type 1, 1

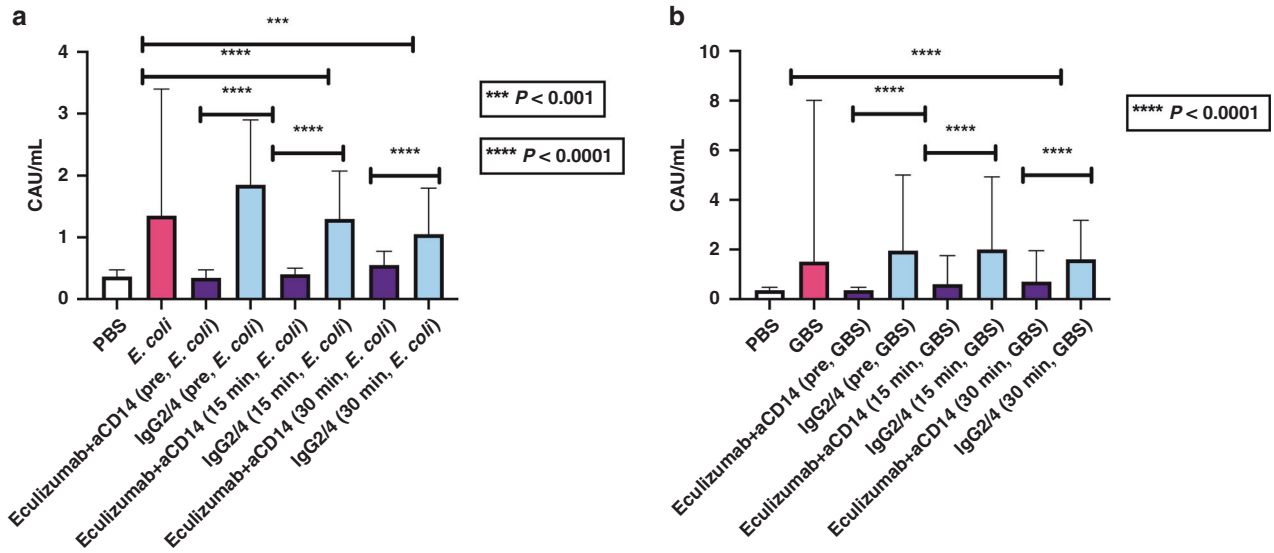

Fig. 3 TCC cord plasma concentrations after 120 min incubation time. Cord plasma concentration of TCC after 120 min incubation time with *E. coli* (**a**) and group B streptococci (**b**), and dual inhibition with complement C5 and co-receptor CD14 inhibitors, pre- and post-bacterial challenge (Sub-study 1; cord blood $n = 16$). Graphic presentations of bar charts with median and IQR values. PBS phosphate-buffered saline, Pre 8 min prior to adding bacteria, 15 and 30 min after adding bacteria, TCC terminal complement complex.

had mild anaemia, 1 had a benign intracranial hypertension, and the other 25 were healthy. Scheduled caesarean delivery was performed at mean (SD) 38.1 ± 0.5 weeks gestation; 18 girls and 12 boys. The mean (SD) birth weight for the 30 babies was 3316 ± 521 g. All Apgar-5 min scores were 9 or 10. No infants were admitted to the neonatal intensive care unit. Mothers in sub-study 2 ($n = 13$) had mean (SD) total white blood count $8.1 \pm 1.6 \times 10^9$/L and mean (SD) neutrophils $5.8 \pm 1.8 \times 10^9$/L. Corresponding values in cord blood ($n = 14$) were mean (SD) total white blood count $12.3 \pm 3.2 \times 10^9$/L and mean (SD) neutrophils $5.9 \pm 2.1 \times 10^9$/L.

Median (IQR) TCC cord plasma concentration after 120 min incubation with *E. coli* was 1.4 (0.7–3.6) CAU/mL, which was similar to concentrations of 1.5 (1.0–9.1) CAU/mL after 120 min incubation with GBS (Fig. 3a, b). The stimulated TCC concentrations were significantly increased compared to negative control at 0.4 (0.1–0.5) CAU/mL ($p < 0.001$). Incubation of bacteria for 240 min in sub-study 2 (Fig. 4a, b) also showed similar TCC cord plasma concentrations with *E. coli* (2.4 [1.5–4.3] CAU/mL) and GBS (1.4 [0.8–3.1] CAU/mL), and significantly higher TCC after bacterial incubation than in the negative controls. Dual inhibition of C5 and CD14 effectively reduced cord TCC plasma concentrations when administered before bacterial challenge, but also when added 15- and 30-min post-challenge after both 120- and 240-min bacterial incubation (Figs. 3 and 4).

Plasma concentrations of TNF, IL-6 and IL-8 after 240 min incubation with *E. coli* or GBS are shown in Fig. 5. There was a significant increase in the plasma concentrations of all cytokines after incubation of cord blood with *E. coli* or GBS (Fig. 5). Incubation with *E. coli* elicited significantly higher TNF, IL-6 and IL-8 levels compared to GBS. Significant reductions in TNF, IL-6 and IL-8 concentrations after the dual inhibition established 8 min prior to, and 15 and 30 min after *E. coli* challenge are summarised in Fig. 5a, c, e. The dual inhibition also resulted in lower crude concentrations of IL-6 after GBS challenge, but differences were not significant (Fig. 5b). For IL-8 and TNF there were no clear effects of dual inhibition after the GBS challenge.

DISCUSSION

This study shows that *E. coli*- and GBS-induced complement activation in cord blood is significantly reduced after dual inhibition of complement C5 and CD14 up to 30 min after bacterial challenge. Moreover, dual C5 and CD14 inhibition in the post-challenge experiments also significantly reduced TNF, IL-6 and IL-8 plasma concentration in cord blood after *E. coli*-induced inflammation. The uniqueness of the ex vivo cord blood model with both complement and cytokine biomarkers makes this study a novel contribution to the understanding of acute innate inflammatory response in EOS, and lay the grounds for further investigations of a potential new adjunctive therapy.

The immediate immune response in neonatal sepsis depends on the multifactorial components, which together make up the innate immune defence system. However, there is still limited data on the impact of the complement system and its role in neonatal inflammation and disease severity.[14] Low concentration of ficolin-3 and mannose binding lectin (MBL), factors activating the lectin complement pathway, have been associated with increased susceptibility to infections.[27,28] In a case-control study, neonates with Gram-positive sepsis had significantly lower ficolin-3 cord blood concentrations than controls, whereas infants with Gram-negative sepsis had lower MBL cord blood concentrations.[27] In contrast, maternally transferred immunoglobulins targeting GBS serotype III can weaken the bacterial capsule, facilitate C3b deposition and thereby enhance complement activation.[7] In our study, we focused on the general activation of the complement system by analysing the TCC concentration, which is regarded to be the best single indicator of complement activation.[29] We found a strong complement activation in cord blood after bacterial challenge with *E. coli* and GBS. High levels of TCC have been observed in adult patients with sepsis complicated by disseminated intravascular coagulation.[30] A factor to consider in our study is that we included mothers and neonates with predominant Caucasian ethnicity. Concentrations of ficolin-3 and MBL can be affected both by intrauterine infections and genetic factors. MBL-deficiency affects e.g. about 30% of the white population.[27]

Neonatal sepsis is associated with an early inflammatory response that is less robust compared to children and adults, but not necessarily less lethal.[31] In our initial experiments (sub-study 1) the bacterial incubation period was 120 min, and we found only a modest cytokine release, in particular after GBS stimulation. A previous cord blood in vitro study reported a

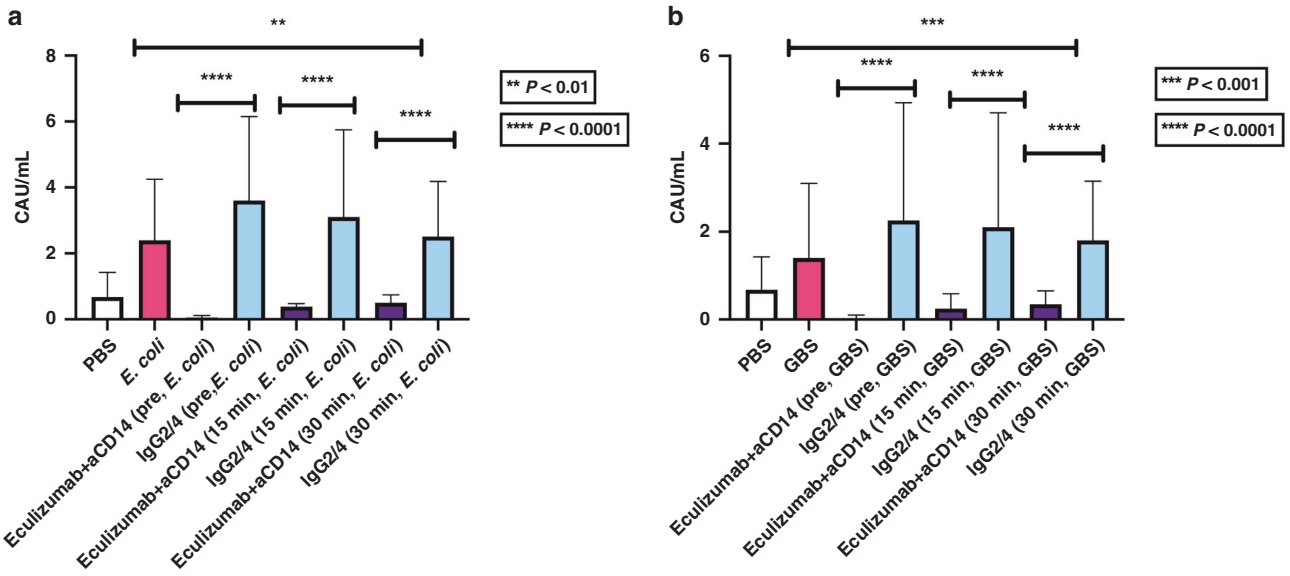

Fig. 4 TCC cord plasma concentrations after 240 min incubation time. Cord plasma concentration of TCC after 240 min incubation time with *E. coli* (**a**) and group B streptococci (**b**), and dual inhibition with complement C5 and co-receptor CD14 inhibitors, pre- and post-bacterial challenge (Sub-study 2; cord blood, $n = 14$). Graphic presentations of bar charts with median and IQR values. PBS phosphate-buffered saline, Pre 8 min prior to adding bacteria, 15 and 30 min after adding bacteria, TCC terminal complement complex.

time-dependent release of proinflammatory cytokines, where TNF was detected first (after 60–120 min), followed by increased concentration of IL-8, IL-6 and IL-1β.[32] We therefore extended the bacterial incubation period to 240 min (sub-study 2) and then detected markedly higher TNF, IL-6 and IL-8 concentrations after bacterial challenge compared to at 120 min. We also found a higher cytokine release after challenge with *E. coli* versus GBS. This stands in contrast to Mohammed et al. who reported no significant difference in cytokine release between the same two bacteria.[32] Other studies also report a high neonatal cytokine release in response to pathogenic *E. coli*, in particular in the preterm infant.[33,34]

There are challenges when comparing our results with previous studies. Many studies report data from in vitro models or studies with isolated blood cells. In our ex vivo model we evaluate the results of the inflammatory crosstalk between a range of cell lines, which is an approach closer to the biology of the innate immune system, and thus theoretically closer to the pathophysiology of neonatal sepsis. Specific factors that may explain variations from other studies are preparation of blood samples, types of stimulators, duration of incubation of blood samples and different assays used for cytokine detection. Experimental studies claiming to mimic EOS vary from in vitro to ex vivo models. In our ex vivo whole blood model we do not claim to have a sepsis model, but a model that reflects the complement activation and release of central pro-inflammatory cytokines induced by bacteria commonly causing EOS in neonates. Using lepirudin as anticoagulant improves the translational value of our results. We found lower cytokine release in our study compared to studies using in vitro models[35–38] suggesting a limitation of the reductionistic in vitro models.[32] This is a further argument to pursue the use of ex vivo models before moving to animal models.[25] We chose the post-challenge approach since it would be more clinically relevant to start empiric therapy after the patient has experienced a septic insult. However, due to limited cord blood we did not include all the timepoints used in our previous post-challenge model.[22] We found that adding inhibitors after 15 and 30 min would be more relevant than already after 5 min. In order to compare our model to previous whole blood models, we also include a pre-challenge timepoint as a reference point. It is common to allow between 5 and 10 min of stabilisation for an active drug before a sepsis challenge,[24,25] so we selected 8 min as the appropriate pre-incubation period in this study.

Activation of the complement system plays an important role in sepsis pathophysiology.[39] Complement acts as a first-line sensor of danger and may accentuate the inflammatory explosion, in an orchestrated effort with other first-line sensors like TLRs.[39] We therefore decided to use an "empiric" approach with dual inhibition of both the C5 molecule of the complement system and CD14, an important co-receptor for TLRs. It is well known that anti-CD14 has no effect on the complement system.[22] However, the dual inhibition of C5 and CD14 was used for all experiments to simplify the protocol, and also because it was not feasible to include separate inhibition studies in the complex experimental set up with limited blood volume. Previous studies have targeted complement C3 because of its central role in the response amplification. However, even though C3 inhibition strongly reduce inflammation, it may also lead to an increased risk of infection by inhibiting the main complement protection through C3-opsonization.[12] Therefore, we chose to use a C5 inhibitor. Inhibition of C5 prevents formation of C5b that induce the assembly of C5b-9 and most importantly, it prevents formation of the potent proinflammatory complement protein C5a. Moreover, this approach does not affect the opsonization of microbes by C3b.[12] Our results are in line with previous studies showing that C5 inhibition significantly reduces TCC plasma concentration in *E. coli*-induced inflammation.[12] Similar to a previous post-challenge whole blood study,[22] we observed a significant inhibition of TCC even when the dual inhibition was added up to 30 minutes after the bacterial challenge, and the effect was similar in *E. coli*- and GBS-induced inflammation. These are promising results for the potential use of C5 inhibition for modulation of the neonatal immune system to reduce sepsis mortality and morbidity. Keshari et al. have already shown that inhibition of C5 protects against organ failure and reduces mortality in a baboon model of *E. coli* sepsis.[40] Another advantage of C5 inhibition is that drugs, such as eculizumab, are already in widespread clinical use, and with relatively good safety data in other paediatric conditions.[41,42] Still, there is some concern that persistent C5 inhibition, due eculizumabs long elimination half-life, potentially may lead to an

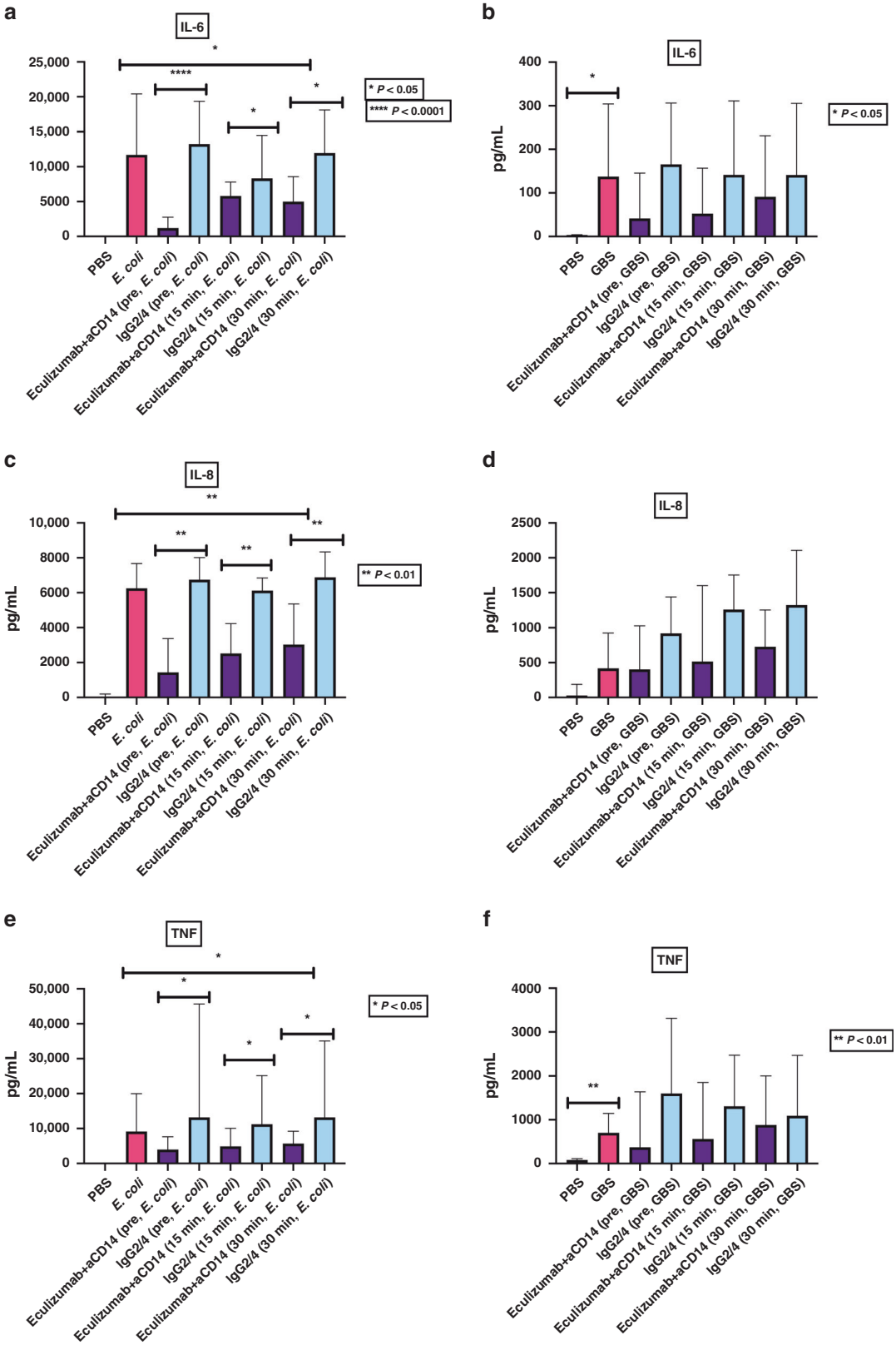

Fig. 5 Cytokine cord plasma concentrations after 120 min incubation time. Cord plasma concentration of IL-6, IL-8 and TNF after 240 min incubation time with *E. coli* (**a**, **c**, **e**) and group B streptococci (**b**, **d**, **f**), and dual inhibition with complement C5 and co-receptor CD14 inhibitors, pre- and post-bacterial challenge. (Sub-study 2; $n = 14$). Graphic presentations of bar charts with median and IQR values. PBS phosphate-buffered saline, Pre 8 min prior to adding bacteria, 15 and 30 min after adding bacteria.

increased risk of subsequent infections. Thus, C5 inhibition in acute sepsis should optimally be treated with C5 inhibitors having shorter elimination half-life.

A previous study has shown that IL-8 release in an LPS-induced inflammation model was significantly reduced by anti-CD14.[43] In our model we showed a significant, but modest reduction of the TNF, IL-6 and IL-8 release after dual inhibition to the *E. coli* challenge. We did not observe any significant reduction in the already quite low cytokine release in response to the GBS challenge. Skjeflo and co-workers showed that the simultaneous inhibition of CD14 and complement efficiently reduced the inflammatory response induced by various strains of *Staphylococcus aureus* in a similar human whole blood model, as used in our experiments.[17] However, in contrary to Gram-negative-induced inflammation, the responses were primarily dependent on complement, whereas CD14 inhibition played a less important role in the Gram-positive *S. aureus* model.[17] Our results point to similar findings for GBS-induced inflammation, and we found no obvious inhibition of cytokine release using the dual inhibition in the GBS-arm of our study. However, animal studies of polymicrobial sepsis have shown clear beneficial effects of the dual C5 and CD14 inhibition with improved hemodynamic parameters, and morbidity and survival, and the dual inhibition may thus be relevant for a broader range of sepsis pathologies.[44,45]

Our study has strengths and limitations. The most obvious strength is the ex vivo model assessing the innate immune response in a system with fresh cord blood containing both cellular and humoral immune response components. We assessed a novel immunological approach with promising results in adult and animal pre-challenge models and found similar potential beneficial effects in the neonatal inflammatory ex vivo model. However, the study also has limitations. First, our model did not allow us to address some of the known intracellular and extracellular bacterial antigen-specific mechanisms which can be done in in vitro experiments.[14] Second, five mothers with well-managed underlying medical conditions were included, but baseline values and post challenge values (complement and cytokines) of these five did not deviate from the remaining 25 included. Third, other studies have used a higher GBS load of 10^8 CFU/mL or extended observation time up to 24 h, and these authors suggested that this is necessary to mimic the first stages of neonatal sepsis.[46] However, a GBS concentration of 10^7 GBS/mL should be adequate, and indeed the median IL-6 value observed after GBS challenge was markedly above a suggested cut-off levels for neonatal sepsis.[47] Fourth, due to limited blood volume and the complexity of the experimental set-up it was not possible to add an experimental part using eculizumab and anti-CD14 as separate and single inhibition agents. Finally, due to the complexity of the experimental protocol and the need to activate the blood within 20 min of sampling we decided to use heat-inactivated bacteria and limit the outcomes analysed, like in other studies.[17,20–23] Previous vaccine studies have shown that heat inactivated GBS elicit a clear immune response, and our findings confirm this.[48] Moreover, heat-inactivated GBS induce TLR2-dependent antimicrobial gene activation,[49] and neither LPS nor LTA are destroyed by the heat inactivation process. However, the heat-inactivated GBS and *E. coli* strains/serotypes used in the current study may not be representative for all GBS and *E. coli* strains that infect neonates.

In conclusion, promising results in this umbilical cord blood inflammation model, using bacteria with clinical relevance for EOS, along with similar findings in previous adult blood models and animal studies indicate that the dual inhibition of C5 and CD14 might be a future approach for treating severe cases of neonatal sepsis. Further experiments in animal models may be the first step to assess this novel strategy.

DATA AVAILABILITY

The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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ACKNOWLEDGEMENTS

We thank all participating mothers for their contributions to this study and the personnel at the Department of Obstetrics at University Hospital of North Norway for important collaboration and help to organise the study.

AUTHOR CONTRIBUTIONS

Substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data: A.U.B., H.N.G., J.P.C., I.H., J.K.L., C.L., T.E., T.E.M., C.K. Drafting the article: A.U.B. and C.K. Critically revised the manuscript and approved the final version to be published: all authors.

FUNDING

The first author was funded by UiT-The Arctic University of Norway as a medical research student.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Regional Ethical Committee (2019/834/REK nord). All participating women gave informed written consent to participate in the study. All participants signed a written consent.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41390-023-02489-2.

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