

SHORT REPORT

Complement C5a is present in CSF of human newborns and is elevated in association with preterm birth

Rozalia Pataký¹ , Forbes A. Howie¹, Guillermina Girardi^{2*}, and James P. Boardman^{1*} 

¹MRC Centre for Reproductive Health, University of Edinburgh, Queen's Medical Research Institute, Edinburgh, UK and ²Department of Women's Health, King's College London, St Thomas' Hospital, London, UK

Abstract

Neuroinflammation contributes to developmental brain injury associated with preterm birth, but the mediators that drive it are incompletely understood. Previous studies have shown that complement C5a is present and injurious in the brains of foetal mice exposed to preterm labour. Here, we demonstrate that C5a is present in the cerebrospinal fluid of newborn human infants and that levels are elevated in those born preterm. The difference is not explained by systemic infection. Complement activation in the neonatal brain and its role as a potential therapeutic target in preterm brain injury warrant further study. Activation in the neonatal brain and its role as a potential therapeutic target for preterm brain injury warrants further study.

Introduction

Preterm birth is strongly associated with a phenotype that includes brain injury and long-term neurodevelopmental and psychiatric impairments. Inflammation is a major contributor to injury but the mediators and receptors involved are uncertain [1].

The complement system is part of the innate immune response to tissue injury and infection. Its four pathways all result in the production of C5a, a potent anaphylatoxin that binds to receptor C5aR to induce a range of inflammatory activities. C5aR is widely expressed in the human central nervous system [2], including in early development [3,4], and C5a-C5aR signalling is implicated in the pathogenesis of neurodegenerative and inflammatory CNS diseases in experimental models [2]. In a mouse model of inflammation-induced preterm birth C5a contributes to cortical neuronal injury *in utero*, mediated through glutamate excitotoxicity [5]. Importantly, in this model, neuronal protection was achieved through C5aR blockade, which raises the possibility that modifying the complement system could be an effective neuroprotective strategy. There are limited data on C5a in human CNS disease and its role in developmental brain injury is unknown. We aimed to determine whether C5a is present in

Keywords

Preterm infant, brain, neuroinflammation, complement system, C5a

History

Received 15 August 2016

Accepted 19 October 2016

the cerebrospinal fluid (CSF) of newborns, and hypothesised that levels would be increased among those born preterm.

Materials and methods

Participants

We recruited two groups of neonates from the Royal Infirmary of Edinburgh between June 2014 and September 2015 who required CSF sampling, usually for the evaluation of suspected sepsis: (1) preterm neonates (<32 weeks' gestation); and control infants born at >37 weeks' gestation. Infants were not eligible if they had a chromosomal abnormality, congenital malformation, or congenital infection.

Blood stream infection (BSI) at the time of CSF sampling was defined as either (1) blood culture grew a pathogenic bacterial species; or (2) the blood culture was negative or grew coagulase negative Staphylococcus (CoNS) and the infant had one or more signs of generalised infection (apnoea, temperature instability, feeding intolerance, worsening respiratory distress or hemodynamic instability) and the attending neonatologist treated with IV antibiotics for ≥ 5 d.

Sequential cranial ultrasound examinations were performed in preterm infants during the NICU stay, and the worst grade of brain injury is reported (Supplemental Table 1).

Written parental informed consent was obtained, and the study was approved by the UK National Research Ethics Service.

CSF samples

Lumbar puncture was carried out using a 22G spinal needle and 4 drops of CSF were collected after clinical samples. The

*These authors contributed equally to this work.

Address for correspondence: Dr James P. Boardman, W1.26 Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK. Tel: +44 131 242 2567. E-mail: james.boardman@ed.ac.uk
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Table 1. Characteristics of neonates.

	Preterms (<i>n</i> = 17)	Term controls (<i>n</i> = 20)
Mean PMA age at birth/weeks (sd)	27.14 (2.14)	39.86 (1.86)
Mean PMA at sample/weeks collection (sd)	29.29 (2.86)	40.29 (2.0)
Day after birth life at sample collection/median (IQR)	10 (6-21)	2 (1-3)
Mean birth weight/g (sd)	1027 (299)	3484 (568)
BSI at the time of sample collection	10 (59%)	8 (40%)
Mechanical ventilation		
Number of infants requiring mechanical ventilation prior to CSF sample	16*	5*
Number of ventilator days/median (IQR)*	1 (1-6)	2 (1-2)
Necrotising enterocolitis prior to CSF sample/number	1	0
Gender (M:F)	13:4	16:4
Antenatal steroid exposure (%)	16 (94%)	0
Antenatal MgSO ₄ (%)	12 (71%)	0

*Reasons for short-term mechanical ventilation in the preterm infants were respiratory distress syndrome or suspected/confirmed bacterial sepsis; and in the term group all five cases were for suspected/confirmed bacterial sepsis and in one of these there was evidence of meconium pneumonitis with air leak.

specimens were put on ice, centrifuged at 1000 rpm at 4 °C for 10 min and the supernatant was frozen at –80 °C for batch analysis.

C5a measurement

Because C5a is rapidly cleaved to metabolite C5adesArg, we measured C5adesArg to estimate C5a levels in thawed CSF samples, using the C5a human ELISA kit (Hycult Biotech, Uden, The Netherlands.), following the instructions of the manufacturer. The between batch coefficient of variation (CV) was 3%; the within batch CV was 3.4% and 2.6% across the two batches; and the lower limit of detection was 0.3 ng/ml.

Data analysis

Pearson's Chi-squared test was used to compare the proportions. The distribution of C5a values was tested for equality of variance using Levene's test, and group differences were investigated using Student's *t*-test. GLM ANOVA was used to investigate possible confounding effects of systemic sepsis. Analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL).

Results

Participants

CSF for C5a measurements was obtained from 20 term and 17 preterm infants (Table 1), and individual participant's blood, CSF and diagnostic information were collected (Supplemental Table 1).

Cerebrospinal fluid analysis

There was no significant difference in the proportion of infants with CSF contaminated by blood defined as RBC > 1000 cells/mm³: 50% preterm versus 42% of controls, *p* = 0.73. The difference in proportion of infants with BSI in each group was not statistically significant (*p* = 0.33), and there was no significant difference in CSF RBC count between infants with and without BSI (*p* = 0.559).

No infant had meningitis. CSF white cell counts were within normal limits for age with the exception of one preterm

infant (PT1) who had a marked CSF lymphocytosis without evidence of congenital infection, viral meningitis or neuro-metabolic disease. This individual had florid intraparenchymal echodensities on cranial ultrasound at the time of CSF sampling, and later MRI revealed evolution of these to cystic PVL. PT1's CSF C5a concentration was 2.162 ng/ml, which was within the upper ×1.5 IQR of values for the preterm group (Figure 1).

C5a in cerebrospinal fluid

C5a was present in the CSF of preterm and term infants, but values were higher in preterms compared with controls: mean CSF C5a concentration in preterm infants was 1.75 ng/ml (range 0.42–5.24) versus 0.98 ng/ml (range 0.37–2.48) in term infants, *p* = 0.006 (Figure 1).

When BSI at the time of sampling was included in the GLM ANOVA, the effect of prematurity remained statistically significant (*F*-statistic 9.3, *p* = 0.005) and BSI was not significant (*F*-statistic 1.23, *p* = 0.28).

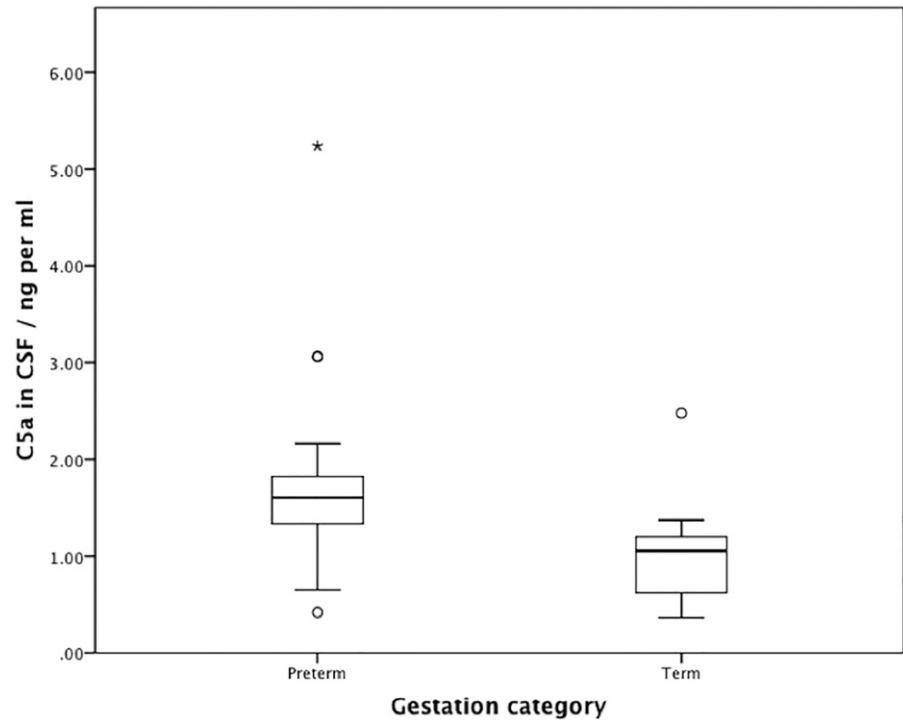
Discussion

We demonstrated an increase in complement component C5a in the CSF of preterm infants compared with infants born at term. Our observations are consistent with the concept of a perinatal inflammatory response in CSF after preterm birth [6], and they provide a new potential therapeutic target for reducing preterm brain injury.

The data build upon studies in mice that implicate the C5a-C5aR pathway in the mechanism leading to increased glutamate release and foetal brain injury associated with intrauterine inflammation [5]. Further supportive evidence for a neurotoxic role of C5a in developmental brain injury comes from the observation that *in utero* exposure to malaria in mice alters brain development and outcome through a C5a-C5aR dependent pathway in a similar manner to the preterm birth mouse model [7].

The median age of sampling the preterm group was day 10 after birth and for the term group it was 2 d. Given the rapid turnover of CSF in the newborn (estimated to be 5 ml/kg/h), it is likely that C5a, which can be synthesised by all central nervous system cell types, is generated in the postnatal period

Figure 1. Boxplot of C5a values in preterm and term infants (bar, median; box, 1st and 3rd quartile; whiskers ± 1.5 IQR).



for both groups. Specific co-morbidities of preterm birth such as respiratory disease requiring mechanical ventilation and necrotising enterocolitis are associated with a systemic inflammatory response, and it is possible that these pathologies as well as blood stream infection sepsis (Table 1), could have contributed to an enhanced C5a response in CNS that was established over a longer period of time in the preterm group. This has clear implications for timing of potential therapies designed to target dysregulated C5a. Further studies with placental histopathology and cord blood sampling would be required to determine whether increased C5a is also a component of the foetal inflammatory response syndrome as suggested by pre-clinical studies.

CSF samples from neonates may contain red blood cells, which is most commonly due to contamination during sampling or intraventricular haemorrhage. There was no difference in the proportion of blood-stained taps (defined as $RBC > 1000/ml$) between the preterm and term groups, and there was no significant difference in the RBC count of CSF samples taken from infants with and without systemic sepsis. Therefore, it is unlikely that sample contamination by blood explains the findings in CSF.

While some studies suggest physiological effects of C5a in foetal brain development [4], many others have demonstrated toxic effects of excessive C5a on neurons, glia and endothelial cells in models of neurodegeneration, CNS injury and CNS infection [2]. C5aR blockade augments the neuroprotective effects of hypothermia in *in vitro* systems of ischaemia [8], and pre-clinical studies show that inhibition of C5a generation with eculizumab or treatment with statins attenuates perinatal brain injury. These agents are already used in humans and they are safe for mother and foetus [9,10].

In conclusion, we provide evidence of a perinatal inflammatory response in CSF with a modest but statistically significant increase the concentration of complement split

product C5a in preterm infants compared to term infants. C5a might be an important therapeutic target to reduce brain injury associated with preterm birth and further investigation is warranted.



Acknowledgements

The authors are grateful to the parents and carers who consented to take part in the study, and to clinical colleagues at the Neonatal Intensive Care Unit of the Royal Infirmary of Edinburgh for referring patients.

Declaration of interest

The authors declare that they have no competing interests. This work was supported by the Theirworld (www.theirworld.org) and was undertaken in the MRC Centre for Reproductive Health which is funded by MRC Centre grant (MRC G1002033).

ORCID

James P. Boardman  <http://orcid.org/0000-0003-3904-8960>
 Rozalia Pataky  <http://orcid.org/0000-0002-7895-0263>

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Supplementary material available online