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# Complement anaphylatoxin C3a and C5a formation in premature children with respiratory distress

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**Abstract** Premature children ($n$ = 25) with respiratory distress (RD) were studied regarding complement activation and formation of the anaphylatoxins C3a and C5a. Blood samples were drawn on admission to the paediatric intensive care unit. In 18 of the patients RD was accompanied by other perinatal complications like pneumothorax or intracerebral haemorrhages. Seven of the premature children had RD without such complications. Preterm children with RD and with peri- and postnatal complications such as pneumothorax or intracerebral haemorrhage had increased concentrations in plasma of the anaphylatoxins C3a and C5a compared with preterm children with RD without these complications. There was a positive correlation between the plasma C3a and C5a concentrations in the preterm children.

**Conclusion** The present study indicates that isolated RD will appear without signs of complement activation and that complications like pneumothorax or intracerebral haemorrhages are associated with release of the anaphylatoxins C3a and C5a.

**Key words** Anaphylatoxin · Complement · Respiratory distress · Preterm children

**Abbreviations** *ARDS* adult respiratory distress syndrome · *ICH* intracranial haemorrhage · *RD* infant respiratory distress syndrome · *TCC* terminal C5b-9 complement complex

## Introduction

Circulatory and respiratory complications are common in premature children [6, 20]. These children often suffer from severe infections and brain haemorrhages [17]. A pathophysiological role of complement activation in these clinical situations has been discussed [6, 11, 20, 26, 28].

When complement is activated, anaphylatoxins (C3a and C5a) are released (Fig. 1). Immune complexes and bacteria activate the classical pathway of the complement cascade [15]. Alternative pathway activation can be initiated by foreign materials or tissue altered by heat, mechanical trauma or hypoperfusion and bacteria [1, 2, 14]. The anaphylatoxins in human serum are rapidly inactivated by an enzyme with carboxypeptidase activity. The serum carboxypeptidase B cleaves off the C-terminal arginine residue from the anaphylatoxins [5]. Thereafter, the $C3a_{desArginine}$ and the $C5a_{desArginine}$ molecules only retain part of their activities. They increase vascular permeability and trigger smooth muscle contraction and release of histamine from mast cells and basophils [15]. Anaphylatoxins may also impair cardiac function [12]. Important interactions exist among the complement, kinin, coagulation and fibrinolytic systems [27]. Both C3a and C5a release proteases, peroxidases and cytokines from leucocytes [4, 9, 24, 30]. Neutrophils and macrophages activated by C5a and $C5a_{desArginine}$ may also cause release of superoxides and of pro-inflammatory cytokines [8]. In adults, prolonged elevation of plasma anaphylatoxins is

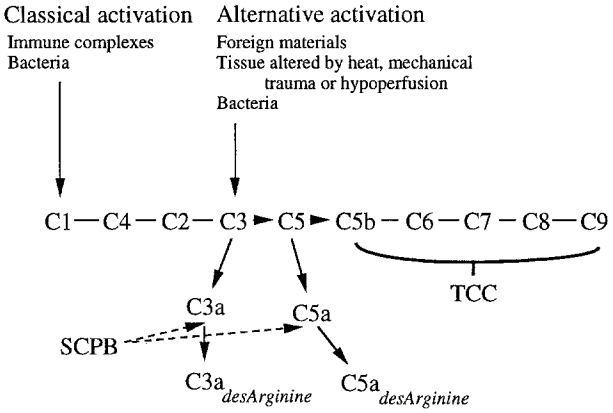


**Fig. 1** A schematic and simplified illustration of the complement cascade. (*SCPB* serum carboxypeptidase B, *TCC* terminal C5b-9 complement complex)

associated with development of adult respiratory distress syndrome (ARDS) and multisystem organ failure [1, 13, 14]. Complement-induced leucocyte activation and granulocyte aggregation has in adults been proposed as a major factor in the aetiology of the ARDS [19, 22].

This study was designed to determine if the complement cascade is activated in premature children with infant respiratory distress syndrome (RD). We hypothesized that if it was there would be a difference in the level of activation between infants with respiratory distress who did and did not develop complications.

## Patients and methods

### Patients

Twenty-five consecutive premature children with RD admitted to the paediatric intensive care unit for ventilator treatment were studied. All patients had RD defined as; tachypnoea, grunting, cyanosis with 40% oxygen and an abnormal chest radiograph appearance.

Seven full-term babies born by caesarean section, with normal Apgar scores, served as a control group.

### Material

The patients were admitted to the paediatric intensive care unit within 24 h after birth and venous blood samples were drawn immediately after arrival. In 2 of the children, blood samples were drawn every day during their stay at the intensive care unit. The protocol was approved by the Ethical Committee for Human Studies of the University of Göteborg and all patients' relatives gave their consent.

Chest X-ray was performed every 2nd day. Ultrasonic investigation for detection of intracerebral haemorrhages (ICH) was performed once a week during the patients stay in the paediatric intensive care unit. Blood samples were collected before the eventual ICH diagnoses were made. The patients were not transfused with fresh frozen plasma before blood samples were collected. However two children were followed with samples every day where fresh frozen plasma was given as indicated in Fig. 3.

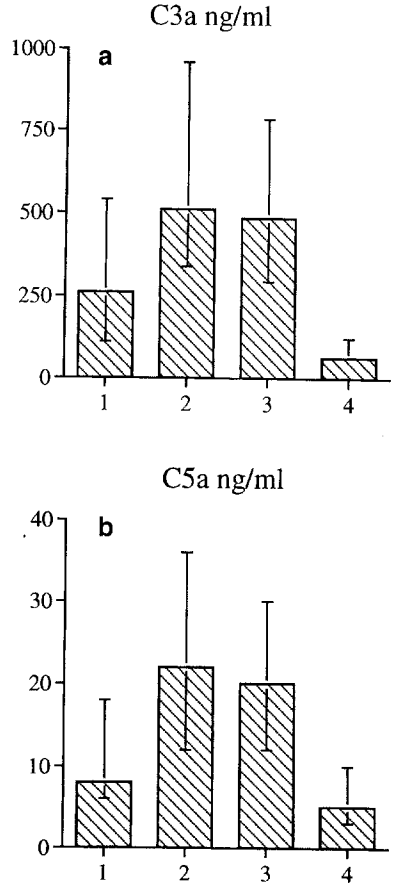


**Fig. 2a, b** Plasma anaphylatoxin (**a** = C3a, **b** = C5a) concentrations in premature children with: *1* RD, *2* RD and pneumothorax, *3* RD and ICH. Number *4* is normal full-term babies born by a caesarean section. Median values and ranges are given

In the control group the blood samples were taken from the umbilical cord vein before the placenta was manually loosened from the uterus and the umbilical cord cut.

### Anaphylatoxin determinations

The blood samples were drawn into tubes with EDTA. The tubes were centrifuged immediately after sampling to remove the cells. The plasma was immediately frozen in separate tubes for each analysis. The tubes were kept at –80° C until the C3a and C5a antigens were assayed. A RIA method was used for these analyses [16, 29]. All quantitations were performed in duplicate. The intraassay coefficient of variation of the duplicate determinations was less than 4% for both C3a and C5a. The interassay coefficient of variation for control samples was less than 6% of the mean for C3a and less than 8% for C5a.

### Statistical analysis

Statistical analyses were performed by calculating the median values, the ranges, the correlation coefficient (by the least square method) and analysis of variance. The Wilcoxon rank sum test was used for analysis of differences between the groups.

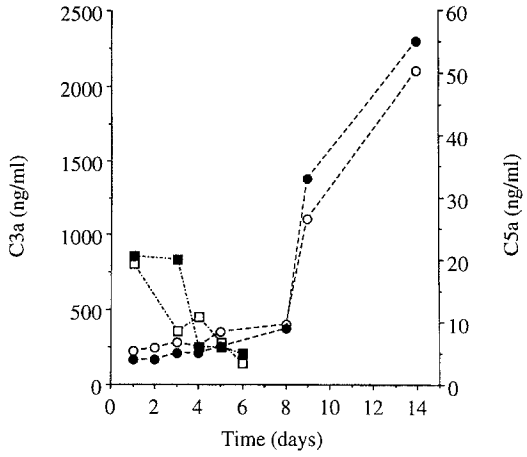


**Fig. 3** A girl born in gestation week 29 (weight 1580 g) with RD. On the 8th day the first ultrasonographic investigation was made and an ICH was diagnosed. She recovered without complications. *Open squares* indicate the C3a values and *filled squares* the C5a values. A premature boy with RD (weight 850 g, gestational age 25 weeks) was diagnosed on the 8th day with an ICH and thereafter required respiratory assistance. He developed severe respiratory insufficiency and died on the 14th day. *Open circles* indicate the C3a values and *filled circles* the C5a values. Both patients were given fresh frozen plasma after the first blood samples were collected

## Results

The plasma concentrations of C3a and C5a on admission to the paediatric intensive care unit in patients with RD combined with and in those without complications like pneumothorax or ICH are given in Figs. 2a and 2b. Premature children with RD accompanied by pneumothorax or ICH had higher plasma C3a and C5a levels than RD children without complications ($P < 0.05$, $P < 0.001$, respectively). There was a positive correlation between C3a and C5a ($r = 0.65$, $n = 27$, $P < 0.05$).

The median gestational age was 31 weeks (range: 26–35 weeks) in patients with only RD and 30 weeks (range: 25–33 weeks) in children with RD associated with complications and 37 weeks (range: 37–38 weeks) in the control group. Corresponding figures for birth weight were 1750, 1470 and 3345 g (ranges: 700–2400, 730–1980 and 2725–3800 g). There were no significant differences regarding gestational age or birth weight between these two RD groups. The median Apgar scores were 7 (range: 5–9) at 1 min and 9 (range: 7–10) at 5 min in patients with uncomplicated RD, and 5 (range: 1–9) at 1 min and 6 (range: 2–10) at 5 min in patients with RD accompanied by pneumothorax or ICH. In the normal group the Apgar scores of all the patients were 9 at 1 min and 10 at 5 min. Seven of the children had RD without signs of pneumothorax or ICH. Eight of the children had pneumothorax and 16 had ICH. The combination of pneumothorax and ICH was found in seven of the preterm children.

Two of the patients with RD and complications followed on a longitudinal basis are shown in Fig. 3. A girl with RD (weight 1580 g, gestational age 29 weeks) was diagnosed with ICH when a ultrasonographic investigation was made on the 8th day. She recovered without complications and the C3a and C5a concentrations in plasma returned to the normal range within 4 days. A premature boy with RD (weight 850 g, gestational age 25 weeks) followed another course. On the 8th day of life an ICH was diagnosed and thereafter the patient required respirator ventilation. He developed severe respiratory insufficiency and died on the 14th day. Plasma concentrations of C3a and C5a increased from the normal range to high levels.

## Discussion

Several authors have demonstrated that elevation of complement anaphylatoxins is associated with ARDS development in polytrauma and septic patients [1, 13, 14, 22, 32]. It has also been demonstrated that the extent of complement activation correlates to the pulmonary shunt and the extravascular lung water [31]. Fetal stress, cardiac insufficiency, asphyxia and lack of surfactant may contribute to development of RD [3, 18]. It is often difficult and even impossible to distinguish between ARDS and RD in the neonate [7, 21, 23]. The inflammatory reaction seems, however, to be a more important factor behind the development of ARDS. Reports have indicated reduced activity of both the classical and the alternative pathways of complement in newborn infants compared with their mothers or adult controls [17]. This reduction has also been discussed as the primary reason behind the increased susceptibility of newborn infants to infection [17]. Although the study by Johnston and co-workers indicates that preterm infants have a low complement activity, the present study indicates that premature children with birth complications have high plasma levels of anaphylatoxins. This is also in line with results of a study by Groneck and co-workers [10]. They demonstrated that increased levels of C5a can be found in tracheobronchial aspirate fluid of preterm infants at risk for lung disease [10]. In another study, Schrod et al. [25] showed that C3a fragments were found in excess in asphyxiated neonates. These two reports suggest a relationship between high levels of anaphylatoxins and RD accompanied by other complications in line with our findings. As the anaphylatoxins induce smooth muscle contraction and increase vascular permeability and histamine release, complement activation may be related to the complications occurring before and after birth.

The anaphylatoxins in adults are inactivated by removal of an arginine molecule. C3a and $C3a_{desArginine}$ are eliminated via the kidneys [15]. C5a in adults is internalized and degraded by neutrophils. C5a and $C5a_{desArginine}$

are not found free in plasma until the neutrophil binding capacity is exceeded [15]. Therefore, it has been difficult to find elevated C5a concentrations in circulating blood even in severely traumatised or septic adults. In the present study of preterm infants, however, increased C5a concentrations were often found. In adults, elevated plasma levels of C5a are very seldom found, and the correlation between C3a and C5a is poor. The terminal C5b-9 complement complex (TCC) is formed as an end product of complement activation. In adults undergoing cardiopulmonary bypass operations, there was a positive correlation between C3a and TCC [2]. However, it was not possible to detect any correlation between C5a and TCC or between C3a and C5a in adults [2]. The correlation found in preterm infants between C3a and C5a, and the high plasma concentrations of C5a may be explained by a limited capacity of the leucocytes to bind C5a and $C5a_{desArginine}$ in preterm infants.

In conclusion, this study demonstrates that in preterm children with RD without pneumothorax or ICH, the anaphylatoxins C3a and C5a are not extensively elevated. In preterm children with RD and with peri and postnatal complications like pneumothorax or ICH, the complement system was activated, with increased concentrations of the complement anaphylatoxins C3a and C5a in plasma.

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