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Complement activation in septic baboons detected by neoepitope-specific assays for C3b/iC3b/C3c, C5a and the terminal C5b-9 complement complex (TCC)

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SUMMARY

We have investigated the cross-reactivity of various species in neoepitope-specific methods for quantification of human complement activation products. In contrast to most other species examined, baboon showed a substantial cross-reactivity supporting a high degree of homology between human and baboon complement. An assay for C3b, iC3b and C3c (MoAb bH6) showed moderately good reactivity, in contrast to a C3a assay which did not cross-react. Excellent reactivity was found for C5a using MoAbs C17/5 and G25/2. The reactivity of an established TCC assay (MoAb aE11 to a C9 neoepitope and polyclonal antibody to C5) was improved substantially by replacing the anti-C5 antibody with a new MoAb to C6 particularly selected on the basis of baboon cross-reactivity. Plasma samples from baboons receiving $2{\cdot}5 \times 10^9$ and $1{\cdot}0 \times 10^{10}$ live *Escherichia coli* bacteria/kg were examined with the assays described. *In vivo* complement activation with the lowest dose was moderate and kept under control, in contrast to the highest dose, where an uncontrolled increase in all activation products continued throughout the infusion period. These results support the hypothesis that sufficiently high amounts of endotoxin lead to uncontrolled activation of complement as seen in irreversible septic shock. The results are discussed with particular emphasis on activation of the terminal complement pathway.

Keywords baboon sepsis *Escherichia coli* complement activation C3 activation C5a terminal complement complex

INTRODUCTION

Endotoxin-induced septic shock is associated with activation of the complement system [1]. The degree of activation has been found to correlate with the prognosis of the disease [2–4]. In humans we have previously shown that the plasma concentration of complement activation products, particularly of the terminal C5b-9 complement complex (TCC), measured on admission to hospital, clearly discriminated between survivors and non-survivors of meningococcal septicaemia [5]. Activation of complement has also been found to contribute to the pathophysiology of the adult respiratory distress syndrome (ARDS) and multiorgan failure (MOF), often seen as complications to septic shock [6–8].

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A series of adverse effects is known to occur when complement is activated. Normal mice were consistently found to have a markedly higher lethality than C5-deficient mice challenged with endotoxin and tumour necrosis factor [9]. Nevertheless, the primary role of complement activation in the pathogenesis of irreversible septic shock remains unclear. Sensitive and specific assays for detection of complement activation products in laboratory animals have been lacking. Novel assays, based on MoAbs to neoepitopes of activation products, are now available for detection of activation at any level of the complement cascade in humans [10].

Infusion of *Escherichia coli* bacteria into baboons is considered to be a good experimental model for human septic shock [11]. Bengtsson *et al.* recently reported that our previously published TCC assay showed cross-reactivity to baboons [12]. The *in vivo* effect of endotoxin on complement activation was investigated by infusing 10^8, 10^9 or 10^{10} live *E. coli* bacteria/kg

body weight. The concentration of TCC increased significantly in animals receiving 10^{10} and 10^9, but not 10^8, bacteria and correlated with the level of elastase and endotoxin. These results led us to investigate other human neoepitope complement activation methods for cross-reactivity and to optimize the TCC method for detection of activated baboon complement.

MATERIALS AND METHODS

General enzyme immunoassay procedure

All complement activation assays were designed as double-antibody enzyme immunoassays (EIAs) using a neoepitope-specific antibody as coat and another antibody to detect the captured antigen. Microtitre plates (Nunc Immunoplate II) were obtained from Nunc (Copenhagen, Denmark). The capture antibody was diluted in PBS and coating was performed at 4°C for at least 16 h. The antigen was diluted in cold PBS containing 0·2% Tween 20 and 10 mM ethylenediaminetetraacetic acid (EDTA) and incubated at 4°C for 1 h. Subsequent antibodies and conjugates were diluted in PBS containing 0·1% Tween 20 and incubated at 37°C for 45 min. The plates were washed four times with PBS containing 0·05% Tween in a Dynawasher (Dynatech Laboratories, Alexandria, VA) four times between each incubation. Horseradish peroxidase (see separate methods below) was used as the enzyme and 2, 2 azino-di (3-ethyl)-benzthiazoline sulphonate (Boehringer Mannheim, Germany) and H_2O_2 as the substrate. The substrate was diluted in sodium acetate buffer, pH 4·0. Colour formation was measured spectrophotometrically at 405 nm using 490 nm as reference (Dynatech Model MR 7000). A standard curve was constructed using a zymosan-activated human serum pool. For all assays, except the C5a assay, the standard was defined to contain 1000 AU (arbitrary units) per millilitre. Standards equlibrated with purified baboon complement activation products are not available, as is the case for most of the human assays as well. The C5a standard was calibrated with purified human C5a and expressed as ng/ml. Samples for evaluation of *in vivo* activation were drawn, processed and stored according to recommended guidelines to prevent *in vitro* activation [13]. All samples were examined in triplicate.

Assay for C3a

Two mouse MoAbs to C3a were kindly donated by PROGEN (Heidelberg, Germany). MoAb PR111049, specific for a neoepitope on human C3a, was diluted 1:1000 and used as capture antibody. Peroxidase-labelled MoAb PR111059 to C3a diluted 1:5000 was used as detection antibody. These antibodies have been extensively characterized previously [14,15].

Assay for C3b, iC3b and C3c

The MoAb bH6 [16] specific for a common neoepitope on C3b, iC3b and C3c was used as capture antibody. Polyclonal rabbit anti-C3c (Behringwerke AG, Marburg, Germany) and finally peroxidase-labelled anti-rabbit immunoglobulin (Amersham International, Amersham, UK) served as detection antibodies. The assay has been described in detail previously [17]. Baboon plasma samples were diluted 1:300.

Assay for C5a

The MoAb C17/5 specific for a C5a neoepitope on C5a and C5adesArg was used as capture antibody. Biotinylated MoAb G25/2 and streptavidin conjugated with peroxidase served as detection system. The assay has been described in detail previously [18]. Baboon plasma samples were diluted 1:2.

Assay for TCC

Several modifications of the previously described TCC assay [19] were used. The MoAb aE11 (subclass IgG2a) [20] specific for a neoepitope in C9 was always used as capture antibody. Three alternative detection systems were tried: (i) rabbit polyclonal anti-C5 (Dako A/S, Glostrup, Denmark), conjugated anti-rabbit immunoglobulin (Amersham); (ii) MoAb to C6 (Quidel, San Diego, CA) which we biotinylated in our laboratory, and streptavidin conjugated with peroxidase; and (iii) a novel MoAb (Clone 9C4, IgG1) to C6 produced in our laboratory (see below) and a subclass-specific anti-mouse IgG1 peroxidase conjugate (Southern Biotechnology Associates, Inc., Birmingham, AL). This final modification of the TCC assay was designed as follows: an IgG fraction of aE11 ascitic fluid was obtained using a Protein G Sepharose 4 column (Pharmacia LKB Biotechnology, Uppsala, Sweden). aE11 was further purified with a Mono Q ion exchange column equilibrated with 30 mM Tris-HCl, pH 8·7, containing 2% 1-butanol. The sample was applied and proteins were eluted with a NaCl gradient in the same buffer. Highly purified aE11 (IgG2a fraction to which anti-IgG1 did not react) eluted at 0·27 M NaCl. This fraction was used as capture antibody. Baboon EDTA plasma was diluted 1:2. Culture supernatant of clone 9C4 was diluted 1:1000 and anti-mouse IgG1 1:4000.

Production of anti-C6 monoclonal antibodies

Purified human C6 (Quidel) was used to immunize BALB/c mice. Spleen cells were fused with the myeloma cell line X63-Ag 8.653 according to the method described by Galfre & Milstein [21]. Screening and selection of clones was done in microtitre plates, based on their reactivity against TCC, since the purpose was to obtain antibodies with high reactivity against C6 when incorporated into the TCC. The clone 9C4 was selected based on its high avidity to both human and baboon TCC. The C6 specificity of the clone was finally confirmed in Western blot tests using purified C6, normal human serum and purified TCC. In all cases, the 9C4 clone reacted with a line corresponding to C6 as confirmed by its molecular weight and reactivity with a known polyclonal anti-C6 antibody (Quidel). SDS–PAGE and Western blot tests were performed according to previously described methods [22,23].

Animals

Five adult male baboons (*Papio ursinus*) weighing 21–25 kg were used. They were allowed to stabilize in the animal facility for 1 month. During this period the animals were tested for tuberculosis as well as parasitic and other diseases. The guidelines provided by the National Research Council Guide for the Care and Use of Laboratory Animals (USA) were closely followed throughout the experiments. The animals were fasted overnight and tranquillized with ketamine hydrochloride (Ketalar) 6–8 mg/kg body weight intramuscularly 1 h in advance of the experiment. Anaesthesia was performed as previously described [11]. The animals breathed spontaneously with a minimum continuous positive airway pressure of 1–2 cm H_2O.

Instrumentation

A tracheotomy was placed (tube size 7–8 F) and the animals were instrumentated for measurements of haemodynamic parameters (arterial and pulmonary catheters). For fluid administration, a catheter was introduced through the femoral and brachial vein of one side (triple lumen catheter). A urinary bladder catheter was placed to measure the hourly urine flow. The pressures were continuously monitored with two Statham P50 pressure transducers (Nikon Kohden).

Preparation of bacteria

Escherichia coli bacteria (organisms RN 2444, type B, ATCC no. 33985) were used. A 1-l Gallenkamp fermenter containing 0·85 *l* of Tryptone Soy Broth was inoculated with a 5% and 18-h-old inoculum of *E. coli* bacteria. The culture then grew for 150 min at 37 C with agitation, aeration and pH control. After 150 min, the culture was centrifuged at 4200 ***g*** for 10 min. The cells were resuspended in sterile physiological saline, centrifuged again and resuspended in the same buffer. Viability was found to be over 90%, calculated as follows. Cells were counted in a Neubauer chamber to obtain total counts, and viable cells were estimated as colony-forming units (CFU). The procedure was performed to maximize living *E. coli* bacteria and to minimize free endotoxin (LPS) levels. Endotoxin levels were determined with a Limulus test (Coatest, Kabi, Sweden) and were found to be equivalent to a total dose of less than 2 μg LPS/kg body weight, even with the 10^{10} CFU/kg dose.

Experimental procedure

Sepsis was induced, after instrumentation and a stabilization period, by infusing live *E. coli* bacteria as previously described [11]. Four baboons received $2{\cdot}5 \times 10^{9}$ (2 h infusion period) and one baboon received $1{\cdot}0 \times 10^{10}$ (8 h infusion period) CFU/kg body weight. After 8 h the animals were killed.

Serum from other species

Serum from 11 other species was kindly provided by The Veterinary Institute, Oslo, Norway. The serum samples, including baboon, were activated with zymosan (10 mg/ml, 1 h, 37 C) and examined in three of the assays described. Cross-reactivity was indicated by the ratio between activated and non-activated serum, arbitrarily scored as negative, poor, moderate and excellent.

RESULTS

Reactivity of baboon in human complement activation assays

Normal baboon serum (NBS) and zymosan-activated baboon serum (ZABS) were examined for cross-reactivity in the following human neoepitope-specific assays for complement activation products: C3a, C3b/iC3b/C3c, C5a and TCC (Fig. 1). No reactivity was found for C3a (Fig. 1a). C3b/iC3b/C3c reacted to some extent (Fig. 1b) but the ratio between NBS and ZABS was

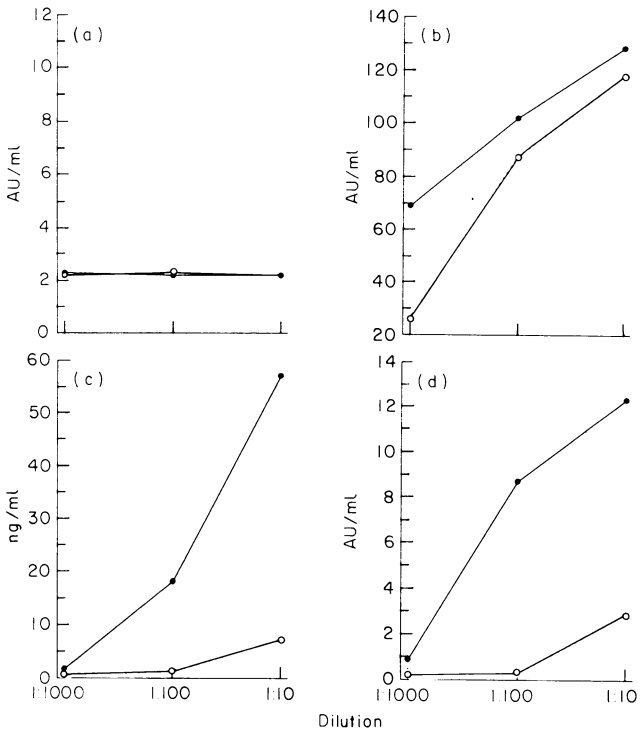

Fig. 1. Reactivity of normal baboon serum (NBS) and zymosan-activated baboon serum (ZABS) in four neoepitope-specific human complement activation assays. No cross-reactivity was found for C3a (a), in contrast to C3b/iC3b/C3c (b). Excellent cross-reactivity was found for C5a (c) and TCC (d). AU, Arbitrary units (for details see Materials and Methods). One representative of three experiments is shown. Each value represents the median of triplicate assays with errors too small to be indicated. ○, NBS; ●, ZABS.

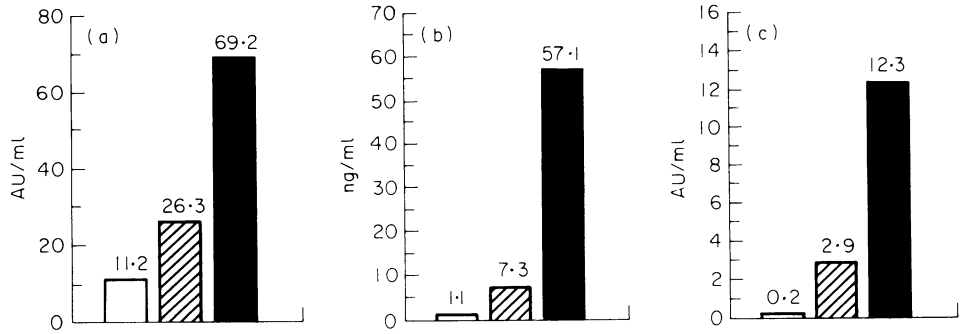

Fig. 2. Reactivity of normal baboon plasma (NBP; □), normal baboon serum (NBS; ▨) and zymosan-activated baboon serum (ZABS; ■) in three neoepitope-specific human complement activation assays. Samples from the experiments shown in Fig. 1 were optimally diluted and EDTA-plasma was included. The best ratio between non-activated and activated samples was obtained for C5a (b), followed by TCC (c) and C3b/iC3b/C3c (a). One representative of three experiments is shown. Each value represents the median of triplicate assays with errors too small to be indicated.

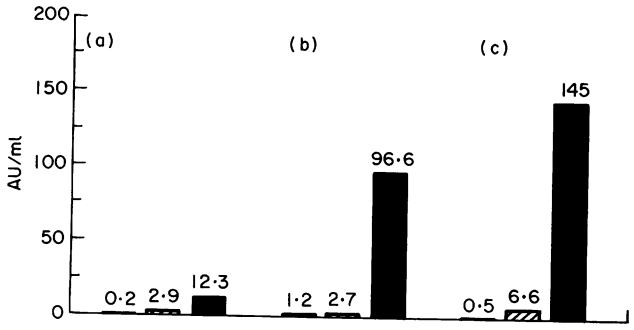

Fig. 3. Reactivity of normal baboon plasma (NBP; □), normal baboon serum (NBS; ■) and zymosan-activated baboon serum (ZABS; ■) in the aE11 TCC assay modified with different detection antibodies. (a) Rabbit polyclonal anti-human C5 (Dako). (b) Mouse monoclonal anti-human C6 (Quidel). (c) Mouse monoclonal anti-human C6 (clone 9C4). One representative of three experiments is shown. Each value represents the median of triplicate assays with errors too small to be indicated.

relatively weak compared with that of C5a and TCC (Fig. 1 c, d). The maximal increase in reactivity after zymosan activation was three times for C3b/iC3b/C3c, in contrast to a 15–20-fold increase for C5a and TCC.

In order to examine *in vivo* activation, the sensitivity of the assays must be sufficient to detect activation products in EDTA-plasma and to reveal the normal gap between plasma and serum. The three assays showing cross-reactivity were therefore tested for normal baboon EDTA-plasma (NBP), NBS and ZABS in optimal dilutions (Fig. 2). A significant difference was observed between NBP and NBS (three to ten-fold) in all assays, reflecting the activation taking place *in vitro* during coagulation [13]. The most satisfactory ratio between NBP, NBS and ZABS was found for C5a, followed by TCC and C3b/iC3b/C3c.

Modification of the TCC assay

In an attempt to improve the cross-reactivity of the TCC assay, we modified the design as described in Materials and Methods.

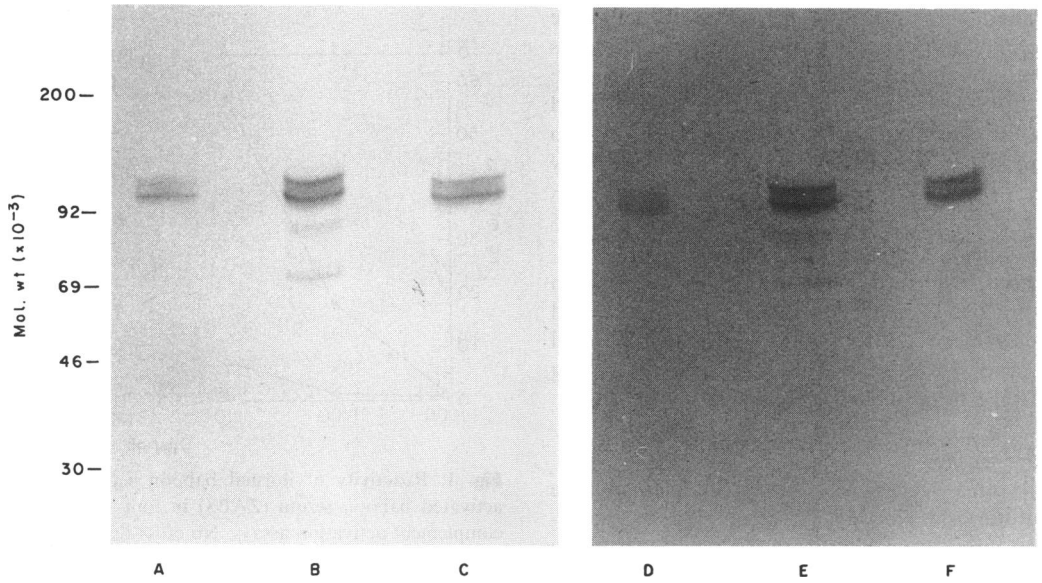

Fig. 4. Reactivity of clone 9C4 in the Western blot test, non-reducing conditions, using 0·1 μl normal human serum (A), 10 ng purified C6 (B) and 100 ng purified TCC (C) as antigen sources. A goat polyclonal anti-human C6 antibody (Quidel) was used as reference with the corresponding antigen sources (D–F).

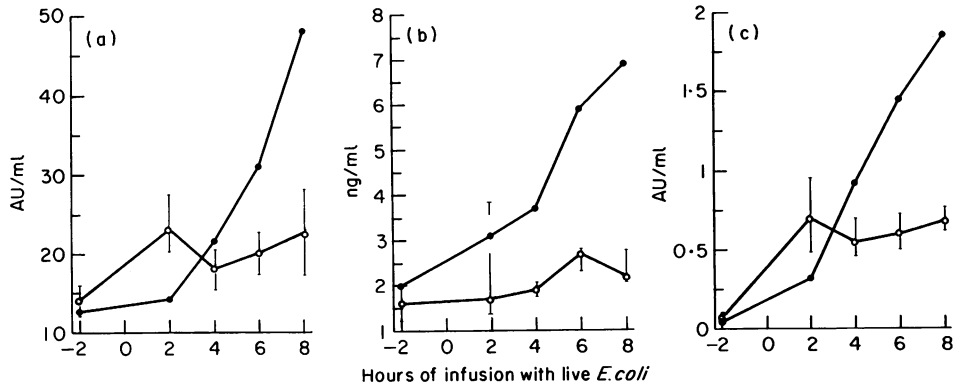

Fig. 5. *In vivo* complement activation in septic baboons. EDTA-plasma samples obtained during infusion of live *Escherichia coli* were examined for C3b/iC3b/C3c (a), C5a (b) and TCC (c). Four baboons received $2{\cdot}5 \times 10^9$ (○) and one baboon received $1{\cdot}0 \times 10^{10}$ (●) bacteria/kg. Each value is the median of triplicate assays. Median and 25–75 percentiles are indicated for the group.

The results are shown in Fig. 3. NBP, NBS and ZABS were examined in the original assay with a polyclonal anti-C5 antibody as detecting antibody (Fig. 3a) and compared with a commercially available anti-C6 MoAb (Fig. 3b). The latter improved the signal considerably. We then produced our own anti-C6 MoAbs of which one (clone 9C4) reacted optimally with both human and baboon TCC (Fig. 3c). The C6 specificity of this clone was confirmed in the Western blot test with purified C6, purified TCC and whole human serum, using a polyclonal anti-C6 antibody as reference (Fig. 4).

In vivo *activation of complement in septic baboons*

Four baboons received $2{\cdot}5 \times 10^9$ and one received $1{\cdot}0 \times 10^{10}$ CFU/kg body weight. EDTA-plasma samples were drawn regularly and examined in the three assays, including the TCC assay modified with clone 9C4 (Fig. 5). With the lowest dose of bacteria, a moderate increase in C3b/iC3b/C3c, C5a and TCC was observed during the first few hours of infusion, after which the level remained stable during the next hours. In contrast, a considerable activation took place with the highest dose of bacteria, with a continuous and steep increase of all activation products during the infusion period.

Cross-reactivity of other species in the activation assays

Eleven other species were tested in the C3b/iC3b/C3c, C5a and TCC assays. The TCC assay used in these experiments was performed with the commercially available anti-C6 MoAb as detection antibody. The cross-reactivity for baboons was excellent compared with the other species. Pig and horse reacted moderately and poorly, respectively, in the TCC assay but not at all in the C3b/iC3b/C3c or C5a assays. No significant cross-reactivity was found for the other species.

DISCUSSION

Lack of reliable methods to detect complement activation in experimental animal models is an important reason for the limited data available on complement as a pathogenetic factor in the development of irreversible septic shock. In a few studies in primates and rats, the anaphylatoxins have been determined and C5a has been found to contribute to the septic shock [24–27]. However, in none of these studies has the novel generation of methods based on neoepitope-specific antibodies been used. These methods have now largely replaced previous assays for reliable detection of complement activation in humans [10]. Some MoAbs to human TCC neoepitopes have been found to cross-react with rabbit, sheep or guinea pig [28] and an assay has been established for quantification of rabbit TCC [29]. Other TCC neoepitope antibodies did not show cross-reactivity against the 33 species tested [30].

In baboons, the assay for human TCC was recently found to be useful for *in vivo* detection of complement activation, whereas the commercially available radioimmunoassays for detecting C3a, C4a and C5a did not react [12]. We have now extended this study and demonstrate in this study three neoepitope-specific assays which cross-react significantly with baboon serum and can be used for *in vivo* evaluation of complement activation at the level of C3, C5 and C9.

The reactivity in the C3b/iC3b/C3c assay was only moderate compared with the C5a assay and the modified TCC assay. Normally, we would expect a much higher degree of activation of C3 than of C5 and C9, both *in vitro* and *in vivo*, due to the relatively more efficient C3 convertase compared with the C5 convertase [31]. Thus, the varying degree of cross-reactivity for the three assays implies that the amount of the various activation products formed should be compared relatively rather than absolutely. Despite the rather low activity obtained with the C3 activation assay, the method could be successfully used for detection of C3 activation *in vivo*.

In humans, it has generally been much more difficult to obtain good and sensitive assays for terminal pathway than for C3 activation. C5a has been particularly difficult to detect *in vivo* due its short half-life by binding to the C5a receptor on neutrophils [32–34]. TCC has been used as an indicator of terminal pathway activation and thus as an indirect indicator of C5a activation [35], since C5 activation *in vivo* is considered to imply release of C5a.

It is important to examine activation of the terminal pathway since C5a is one of the most biologically potent mediators released during activation [36]. In addition to the direct effects induced by the peptide itself, a series of secondary effects occur due to leucocyte and platelet stimulation. These include release of cytokines, reactive oxygen metabolites, histamine, arachidonic acid metabolites and lysosomal enzymes as well as increased expression of various cellular receptors [37–39]. Several of these effects have also recently been demonstrated for TCC when incorporated as C5b-9 (m) in the cell membrane [40].

The present C5a assay, combined with the modified TCC assay, seems to serve as an important tool for surveying terminal pathway activation in septic baboons. The results obtained support the hypothesis that a certain amount of endotoxin may activate complement to a degree which brings the regulatory mechanisms out of control. This may contribute to the disturbed homeostasis seen in irreversible septic shock. Finally, the present methods will be important in the evaluation of the effect on complement activation of various therapeutic interventions in experimental septic shock.

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REFERENCES

1 Padalino P, Gardinali M, Pallavicini J, Chiara O, Bisiani G, Nespoli A. Complement activation and endotoxin in sepsis. Prog Clin Biol Res 1989; **308**:277–82.

2 Hack CE, Nuijens JH, Felt Bersma RJ *et al.* Elevated plasma levels of the anaphylatoxins C3a and C4a are associated with a fatal outcome in sepsis. Am J Med 1989; **86**:20–26.

3 Langlois PF, Gawryl MS. Accentuated formation of the terminal C5b-9 complement complex in patient plasma precedes development of the adult respiratory distress syndrome. Am Rev Respir Dis 1988; **138**:368–75.

4 Zilow G, Sturm JA, Rother U, Kirschfink M. Complement activation and the prognostic value of C3a in patients at risk of adult respiratory distress syndrome. Clin Exp Immunol 1990; **79**:151–7.

5 Brandtzaeg P, Mollnes TE, Kierulf P. Complement activation and endotoxin levels in systemic meningococcal disease. J Infect Dis 1989; **160**:58–65.

6 Swank DW, Moore SB. Roles of the neutrophils and other mediators in adult respiratory distress syndrome. Mayo Clin Proc 1989; **64**:1118–32.

7 Robbins RA, Russ WD, Rasmussen JK, Clayton MM. Activation of the complement system in the adult respiratory distress syndrome. Am Rev Respir Dis 1987; **135**:651–8.

8 Fein AM, Lippman M, Holtzman H, Eliraz A, Goldberg SK. The risk factors, incidence, and prognosis of ARDS following septicemia. Chest 1983; **83**:40–42.

9 Hsueh W, Sun X, Rioja LN, Gonzalez Crussi F. The role of the complement system in shock and tissue injury induced by tumour necrosis factor and endotoxin. Immunology 1990; **70**:309–14.

10 Mollnes TE. Antigenic changes of complement components: relevance for the analysis of complement activation. Complement Inflamm 1989; **6**: 134–41.

11 Schlag G, Redl H, Hallström S, Radmore K, Davies J. Hyperdynamic sepsis in baboons. I. Aspects of hemodynamics. Circ Shock 1991; **34**:311–8.

12 Bengtsson A, Redl H, Paul E, Schlag G, Mollnes TE, Davies J. Complement and leukocyte activation in septic baboons. Circ Shock 1992, in press.

13 Mollnes TE, Garred P, Bergseth G. Effect of time, temperature and anticoagulants on *in vitro* complement activation: consequences for collection and preservation of samples to be examined for complement activation. Clin Exp Immunol 1988; **73**:484–8.

14 Burger R, Zilow G, Bader A, Friedlein A, Naser W. The C terminus of the anaphylatoxin C3a generated upon complement activation represents a neoantigenic determinant with diagnostic potential. J Immunol 1988; **141**:553–8.

15 Zilow G, Naser W, Rutz R, Burger R. Quantitation of the anaphylatoxin C3a in the presence of C3 by a novel sandwich ELISA using monoclonal antibody to a C3a neoepitope. J Immunol Methods 1989; **121**:261–8.

16 Garred P, Mollnes TE, Lea T, Fischer E. Characterization of a monoclonal antibody MoAb bH6 reacting with a neoepitope of human C3 expressed on C3b, iC3b, and C3c. Scand J Immunol 1988; **27**:319–27.

17 Garred P, Mollnes TE, Lea T. Quantification in enzyme-linked immunosorbent assay of a C3 neoepitope expressed on activated human complement factor C3. Scand J Immunol 1988;**27**:329–35.

18 Oppermann M, Schulze M, Gotze O. A sensitive enzyme immunoassay for the quantitation of human C5a/C5a (desArg) anaphylatoxin using a monoclonal antibody with specificity for a neoepitope. Complement Inflamm 1991; **8**:13–24.

19 Mollnes TE, Lea T, Froland SS, Harboe M. Quantification of the terminal complement complex in human plasma by an enzyme-linked immunosorbent assay based on monoclonal antibodies against a neoantigen of the complex. Scand J Immunol 1985; **22**:197–202.

20 Mollnes TE, Lea T, Harboe M, Tschopp J. Monoclonal antibodies recognizing a neoantigen of poly (C9) detect the human terminal complement complex in tissue and plasma. Scand J Immunol 1985; **22**:183–95.

21 Galfre G, Milstein C. Preparation of monoclonal antibodies; stratergies and procedures. In: Langone JJ, Van Vunatis H, eds. Methods in enzymology, vol. 73. New York: Academic Press 1981: 3–46.

22 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; **227**:680–5.

23 Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacryl amide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979; **76**:4350–3.

24 Hangen DH, Bloom RJ, Stevens JH *et al.* Adult respiratory distress syndrome. A live *E. coli* septic primate model. Am J Pathol 1987; **126**:396–400.

25 Hangen DH, Stevens JH, Satoh PS, Hall EW, O Hanley PT, Raffin T. Complement levels in septic primates treated with anti-C5a antibodies. J Surg Res 1989; **46**:195–9.

26 Stevens JH, O'Hanley P, Shapiro JM *et al.* Effects of anti-C5a antibodies on the adult respiratory distress syndrome in septic primates. J Clin Invest 1986; **77**:1812–6.

27 Smedegard G, Cui LX, Hugli TE. Endotoxin-induced shock in the rat. A role for C5a. Am J Pathol 1989; **135**:489–97.

28 Hugo F, Jenne D, Bhakdi S. Monoclonal antibodies against neoantigens of the terminal C5b-9 complex of human complement. Biosci Rep 1985; **5**:649–58.

29 Hugo F, Berstecher C, Kramer S, Fassbender W, Bhakdi S. *In vivo* clearance studies of the terminal fluid-phase complement complex in rabbits. Clin Exp Immunol 1989; **77**:112–6.

30 Wurzner R, Xu H, Franzke A, Schulze M, Peters JH, Gotze O. Blood dendritic cells carry terminal complement complexes on their cell surface as detected by newly developed neoepitope-specific monoclonal antibodies. Immunology 1991; **74**:132–8.

31 Bhakdi S, Fassbender W, Hugo F *et al.* Relative inefficiency of terminal complement activation. J Immunol 1988; **141**:3117–22.

32 Weisdorf DJ, Hammerschmidt DE, Jacob HS, Craddock PR. Rapid *in vivo* clearance of C5a (des arg): a protective mechanism against immune tissue injury. J Lab Clin Med 1981; **98**:823–30.

33 Chenoweth DE, Hugli TE. Demonstration of specific C5a receptor on intact human polymorphonuclear leucocytes. Proc Natl Acad Sci USA 1978; **75**:3943–7.

34 Wagner JL, Hugli TE. Radioimmunoassay for anaphylatoxins: a sensitive method for determining complement activation products in biological fluids. Anal Biochem 1984; **136**:75–88.

35 Mollnes TE, Videm V, Gotze O, Harboe M, Oppermann M. Formation of C5a during cardiopulmonary bypass–inhibition by precoating with heparin. Ann Thorac Surg 1991; **52**:92–97.

36 Hugli TE. Biochemistry and biology of anaphylatoxins. Complement 1986; **3**:111–27.

37 Vogt W. Anaphylatoxins: possible roles in disease. Complement 1986; **3**:177–88.

38 Goodman MG, Chenoweth DE, Weigle WO. Induction of interleukin 1 secretion and enhancement of humoral immunity by binding of human C5a to macophage surface C5a receptors. J Exp Med 1982; **156**:912–7.

39 Scholz W, McClurg MR, Cardenas GJ *et al.* C5a-mediated release of interleukin 6 by human monocytes. Clin Immunol Immunopathol 1990; **57**:297–307.

40 Morgan BP. Complement membrane attack on nucleated cells: resistance, recovery and non-lethal effects. Biochem J 1989; **264**:1–14.