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Involvement of complement pathways in patients with bacterial septicemia

Chantal Dumestre-Pérard [a,*], Elke Doerr [a], Maurice G. Colomb [b], Michael Loos [a]

[a] *Institute of Medical Microbiology and Hygiene, Johannes Gutenberg-University, Hochhaus am Augustusplatz, 55101 Mainz, Germany*
[b] *Université Joseph Fourier, BP53, 38041 Grenoble Cedex 9, France*

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Abstract

The complement system is a major humoral portion of the innate immune system, playing a significant role in host defence against microorganisms. The biological importance of this system is underlined by the fact that at least three different pathways for its activation exist, the classical, the MBL and the alternative pathway. To elucidate the involvement of the classical and/or the MBL pathway during bacterial septicemia, 32 patients with gram-positive and 30 patients with gram-negative bacterial infections were investigated. In patients with gram-positive bacteria, a significant consumption of C1q ($p = 0.005$) but not of mannose-binding lectin (MBL) ($p = 0.2$) was found during the acute phase of infection. In contrast, in patients with gram-negative bacterial infections, a significant reduction of MBL ($p = 0.002$) and only a moderate, less significant reduction of C1q ($p = 0.03$) were observed. As a model for the binding of MBL to gram-negative bacteria, *Salmonella* strains with defined mutations in their lipopolysaccharide (LPS) structure were used. The comparison of the binding of MBL to these *Salmonella* strains with that of the corresponding isolated LPS forms bound to microtiter plates revealed a similar binding pattern, supporting the interpretation that LPS on the surface of gram-negative bacteria is the major acceptor molecule for MBL on these bacteria, which according to our results obviously also takes place during gram-negative bacterial septicaemia. Furthermore, we were able to demonstrate that MBL bound to LPS was able to initiate activation of the complement cascade as measured by the occurrence of the cleavage product C4c.

Keywords: Complement; Mannose-binding lectin (MBL); Bacterial sepsis; Classical pathway; MBL pathway; Lipopolysaccharide (LPS)

1. Introduction

The innate immune defence is composed of humoral and cellular components, which cooperate to eliminate the pathogenic microorganisms in the case of infections. Complement represents one actor of this significant host defence system involved in the immediate protection of the host from bacteria (Song et al., 2000). Recognition of bacteria by complement components is likely to induce the activation of three complement pathways, the classical, the lectin and/or the alternative pathways. All three pathways lead to the formation of a C3 convertase and the generation of complement activation products triggering diverse biological activities, such as opsonization, endocytosis, cytolysis and inflammation, resulting in the elimination of pathogenic microorganisms (Petersen et al., 2001; Reid et al., 2002).

C1q can bind directly to bacteria or indirectly to immune complexes containing bacterial antigen, initiating activation of the classical pathway (Clas and Loos, 1981; Boes et al., 1998), whereas the mannose-binding lectin (MBL) and the alternative pathways are activated directly by bacterial surface components.

MBL carbohydrate recognition domains (CRD) are able to bind, in a calcium-dependent manner, to various carbohydrate structures like mannose, *N*-acetyl-glucosamine, L-fucose and *N*-acetyl-mannosamine present on different microorganisms. However, the binding of MBL to complex carbohydrates structures is poorly understood. The bacterial capsule was reported to reduce MBL binding of several bacteria (Van Emmerik et al., 1994), whereas the structure of the lipopolysaccharide (LPS) or lipooligosaccharide (LOS) plays a role in the MBL attachment to various gram-negative organisms (Devyatyarova-Johnson et

Abbreviations: MBL, mannose-binding lectin; CRD, carbohydrate recognition domain; CRP, C-reactive protein; LPS, lipopolysaccharide; mAb, monoclonal antibody

* Corresponding author at: CNRS FRE 2685, Université Joseph Fourier, Campus Santé, Institut Jean Roget, Domaine de la Merci, BP 170, 38042 Grenoble cedex 9, France. Tel.: +33 4 76 63 71 45; fax: +33 4 76 63 71 23.
E-mail address: Chantal.Dumestre@ujf-grenoble.fr (C. Dumestre-Pérard).

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al., 2000; Jack et al., 2001a,b; Polotsky et al., 1996). Moreover, LPS isolated from the outer cell wall of gram-negative bacteria has been shown to interact with the complement system in different ways (Clas and Loos, 1981).

Several studies have shown a link between low serum concentration of MBL, MBL polymorphism and an increased risk of microbial infection (Turner, 1998; Ezekowitz, 2001). But the involvement of complement in the course of bacterial sepsis has been poorly documented. In mice, the classical pathway was found to be the main complement pathway contributing to innate immunity to *Streptococcus pneumoniae* infection (Brown et al., 2002) and its role was also demonstrated in experimentally induced polymicrobial peritonitis (Celik et al., 2001). In contrast, Zhao et al. (2002) suggested that the O-antigen region of LPS can activate the MBL pathway, which has been shown to play a critical role in a rapid shock response in mice.

The goal of this study was to elucidate the involvement of the different pathways of complement activation during septicemia caused either by gram-positive or by gram-negative bacteria. For the first time, a time-kinetic was performed studying samples from individual patients taken immediately after admission to hospital and through to recovery. Furthermore, the binding of purified MBL to different defined LPS chemotypes of *Salmonella* strains was compared with that of the isolated LPS forms bound to microtiter plates (solid phase).

2. Materials and methods

2.1. Patients and samples

Sixty-two patients with bacterial septicemia, admitted to the Johannes Gutenberg-University Teaching Hospital, were included in the study. Sepsis was diagnosed on the basis of a positive blood culture with isolated and characterised bacteria at the Institute of Medical Microbiology, as well as hyperthermia and elevated concentration of C-reactive protein (CRP). Out of 62 patients, gram-positive bacteria from 32 patients and gram-negative bacteria from 30 patients were isolated. Patients with the following microorganisms were identified: 9 patients with *Staphylococcus aureus*, 17 with *coagulase negative Staphylococcus*, 6 with *Streptococcus* spp., 23 with Enterobacteriaceae (14 *Escherichia coli*, 3 *Enterobacter cloacae*, 2 *Salmonella enterica serovar Enteritidis*, 2 *Klebsiella pneumoniae*, 1 *Yersinia enterocolitica* and 1 *Serratia marcescens*) and 7 with Pseudomonaceae (2 *Acinetobacter lwoffii*, 2 *Pseudomonas aeruginosa* and 3 *Stenotrophomonas maltophilia*).

Serum samples were collected from patients with bacterial septicaemia during the time course of the disease. The first blood sample was taken immediately after submission, after evidence of a positive blood-culture, during the acute phase of sepsis (hyperthermia and high CRP) and subsequent blood samples were taken daily up to the patients' recovery (normal temperature and low CRP).

One patient hospitalised at the haematology clinic for chemotherapy treatment acquired a bacterial septicemia with *Streptococcus sanguis*. In this case, samples were available before, during and after the septicemia phase.

2.2. Bacteria and LPS

The wild-type form (S) and core-deficient mutants Ra, Rb, Rc, Rd1, Rd2 and Re of *Salmonella enterica serovar Minnesota* and the S, Ra, Rb2, Rb3, Rc, Rd1 and Re forms of *Salmonella enterica serovar Tyhimurium* were kindly provided by Dr. G. Schmidt, Borstel, Germany (Clas and Loos, 1981). The bacteria cultured on agar plates were grown in Luria-Bertani (LB) medium (10 g/l tryptone, 5 g/l yeast extract, 5 g/l NaCl, pH 7.4) for 4 h at 37 °C. Afterwards, the microorganisms were centrifuged, resuspended in imidazol-buffered saline (IBS: 150 mM NaCl, 5 mM imidazol, 1 g/l gelatin, 1 mM $MgCl_2$, pH 7.3), counted in a Neubauer chamber, and adjusted to a final concentration of 2×10^9 bacteria/ml. In the case of binding assays with MBL, IBS was supplemented with 20 mM $CaCl_2$.

The isolated LPS preparations of the S, Ra, Rb, Rc and Rd forms from *Salmonella enterica serovar Minnesota* were purchased from List Biological Laboratories (Campbell, USA) and for the Re form of this *Salmonella* strain from Sigma–Aldrich, Steinheim, Germany. Fig. 1 shows the correlation of the polysaccharide composition of LPS from various chemotypes of *Salmonella* according to Ihara et al. (1982).

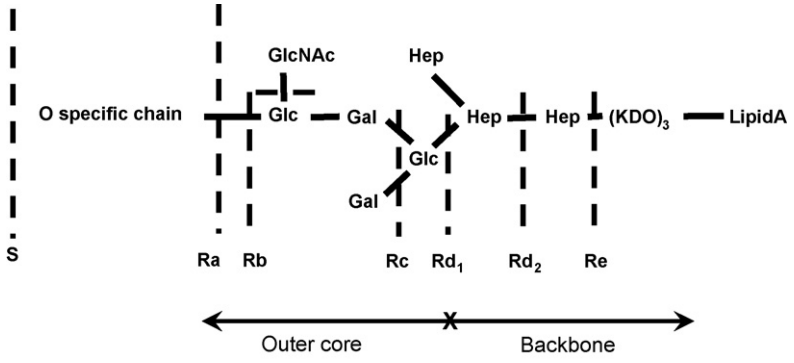

Fig. 1. Polysaccharide composition of LPS from various chemotypes of *Salmonella* (Ihara et al., 1982). GlcNAc, α-*N*-acetyl-D-glucosamine; Glc, α-D-glucose; Gal, α-D-galactose; Hep, L-glycero-α-D-mannoheptose; KDO, 2-keto-3-deoxy-D-mannooctonic acid.