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Components of the lectin pathway of complement activation in paediatric patients of intensive care units

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ABSTRACT

Infections are a major cause of childhood mortality. We investigated components of the lectin pathway of complement activation in the context of sepsis at both genetic and protein levels in neonates, infants and older children. Major components of the lectin pathway and two genes for Toll-like receptors were studied in 87 neonates with confirmed sepsis and compared with 40 babies with infections who did not develop sepsis (disease controls) and 273 infection-free neonatal controls. A second cohort comprised 47 older children with sepsis and 87 controls. Low MBL-conferring genotypes (LXA/O + O/O) were more frequent in sepsis patients than in healthy controls but no significant differences in the frequency of SNPs of other lectin pathway genes (*FCN1*, *FCN2*, *FCN3*, *MASP1/3*, *MASP2*) or TLR receptor genes (*TLR2*, *TLR4*) were found. One case of primary MASP-2 deficiency was found among healthy pre-terms and one neonate suffering from SIRS was heterozygous for the rare *FCN1* gene mutation, +6658 G > A. Generally, sepsis was associated with low serum MBL and low ficolin-2 concentrations on admission. Among neonates, ficolin-1 and MASP-2 levels were elevated in sepsis relative to healthy, but not disease, controls. Unlike neonates, ficolin-3 and MASP-2 levels were lower in older patients than in healthy controls while no difference was found for ficolin-1. With the possible exception of MBL, inherited lectin pathway insufficiencies do not seem to predispose to sepsis, rather changes in protein concentrations reflect alterations in disease course.

1. Introduction

According to the World Health Organization, there were more than 2.8 million neonatal deaths in 2012 globally (approx. 20/1000 live births). Prenatal and perinatal infections, especially when leading to the development of sepsis, are a major cause of neonatal mortality despite progress in prophylaxis and in medical care and treatment (Grimaldi et al., 2014). Furthermore, in survivors, sepsis may lead to irreversible damage of organs or developmental disorders (Sharma et al., 2012). Paediatric sepsis generally is characterized by high risk of fatal outcome or severe complications, although the mortality rate is lower than in adult patients (Thompson and Macias, 2015). The overall mortality from severe sepsis/septic shock in children from developed countries usually does not exceed 10%, but in some countries it may reach 50%. It is estimated that 60–80% of deaths of children aged <5 years (7.5

Abbreviations: MASP, mannose-binding lectin-associated serine protease; MBL, mannose-binding lectin; SIRS, systemic inflammatory response syndrome.

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million deaths yearly) result from sepsis (Khilanani et al., 2014). It should be stressed that accurate diagnosis may be difficult in its early stages (Thompson and Macias, 2015).

During the neonatal period, the innate immune response is believed to play a key role in anti-microbial defence, including the antibody-independent (lectin and alternative) pathways of complement activation. Although the concentrations and activities of complement factors are relatively low, and thus the opsonic and bactericidal activity of serum is less efficient (Sharma et al., 2012; Cedzynski et al., 2012; Milic, 2010), complement activation remains a pivotal mechanism of innate immunity. Moreover, Toll-like receptor (TLR) activity is attenuated but not abolished. For example, expression of TLR4, crucial for the response to Gram-negative bacterial infection/endotoxin and tolerance to alimentary tract commensal flora, is lower in neonates than in older children or adults (reviewed by Sharma et al., 2012 and Cuenca et al., 2013). It seems, therefore that although such innate immune mechanisms are poorly developed in neonates (especially premature babies), they are still of importance and may sometimes make the difference between life and death. Although older children are generally more immunologically competent, innate immunity is still important.

The component of the lectin pathway (LP) of complement activation most widely studied with regard to neonatal sepsis and paediatric sepsis generally is MBL, but findings have been inconsistent and inconclusive. Several reports found low serum MBL concentrations to be a risk factor for development of neonatal sepsis (De Benedetti et al., 2007; Dzwonek et al., 2008; Frakking et al., 2008; Koroglu et al., 2010; Ozkan et al., 2012; Wahab Mohamed and Saeed, 2012; Cakmak et al., 2012). Schlapbach et al. (2010) demonstrated an association of low serum MBL with Gram-negative but not Gram-positive sepsis. On the other hand, some authors found no impact of MBL deficiency on nosocomial invasive infections in newborns (Van der Zwet et al., 2008; Auriti et al., 2010; Aydemir et al., 2011). We ourselves have previously reported an association of low MBL-conferring genotypes (LXA/O + O/O) with neonatal infections in general (Swierzko et al., 2009a) and have studied various lectin pathway components in relation to gestational age, birthweight and incidence of perinatal infections (reviewed by Cedzynski et al., 2012). Only a few studies have described lectin pathway factors in sepsis affecting older children. Fidler et al. (2004) and Agbeko et al. (2010) reported MBL deficiency to be a risk factor.

Here we now report an in-depth study concerning relevant lectin pathway components in the context of paediatric sepsis at both genetic and protein levels. In addition, since the modulatory effect of LP factors on TLR-dependent cell activation has been reported (Wang et al., 2011, 2013; Michalski et al., 2015), single nucleotide polymorphisms of two genes for Toll-like receptors were investigated (*TLR4*, *TLR2*). Patients diagnosed with sepsis were compared both with healthy babies and babies who had less invasive perinatal infections.

2. Material and methods

2.1. Patients and controls

Eighty-seven neonates (mean gestational age 34 weeks; range 24–42) with confirmed sepsis (S1 group) were recruited from the Chair of Neonatology, Poznan University of Medical Sciences (Poland) and Department of Anesthesiology and Intensive Therapy, Polish Mother's Memorial Hospital Research Institute (Lodz, Poland). A further 28 infants aged 1 month to 1 year (mean age 4.4 months) and 19 older children (1–17 years; mean 8.1) were designated group S2. They came from the Department of Anesthesiology and Intensive Therapy, Polish Mother's Memorial Hospital Research Institute. All patients (including neonatal disease controls) were recruited between 2009 and 2013. Sepsis was diagnosed on the basis of clinical criteria specified by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (Goldstein et al., 2005) and recommendations of the Polish Working Group on Sepsis. In brief, diagnosis was based on clinical suspicion supported by body temperature, heart rate, and age-dependent degrees of hypotension and hyperventilation. Some patients could be further classified as having severe sepsis (5 neonatal patients and 8 within S2 group), systemic inflammatory response syndrome (SIRS; $n = 30$ and $n = 7$, respectively) or septic shock ($n = 13$ and $n = 14$, respectively). For most patients, the causative organism(s) was (were) identified (Table 1). Samples were always taken on admission to the intensive care unit and used for the majority of analyses. Serum samples from 68 patients were taken repeatedly (2–27 samples) (Kilpatrick et al., 2015) to estimate changes of concentrations/activities of lectin pathway factors and their correlations with such disease markers as C-reactive protein (CRP), procalcitonin (PCT), fibrinogen (FBG) and blood morphology (white blood cells (WBC) count).

Table 1
Aetiological agents (specified when isolated from blood only) in (A) neonates (S1) and (B) infants and older children (S2).

A

Clinical isolates		Number of cases
Gram-positive bacteria	*Staphylococcus epidermidis*	14 (3 MR[c])
	Staphylococcus haemolyticus	9 (3 MR[c])
	Staphylococcus faecalis	1
	Staphylococcus sp.; coagulase-negative	1
	Streptococcus agalactiae	3
	Streptococcus viridans	2
	Micrococcus sp.	1
	Enterococcus faecalis	2
Gram-negative bacteria	*Escherichia coli*	5
	Pseudomonas aeruginosa	1
>1 agent		7[a]
Fungi	*Candida albicans*	1
Negative blood culture/lack of identification		10

B

Clinical isolates		Number of cases
Gram-positive bacteria	*Staphylococcus aureus*	2 (1 MR[c])
	Staphylococcus epidermidis	4
	Staphylococcus haemolyticus	2
	Staphylococcus hominis	1
	Staphylococcus sp.; coagulase-negative	1
	Lactococcus garviae	1
Gram-negative bacteria	*Acinetobacter* sp.	1
	Stenotrophomonas maltophila	1
>1 agent		7[b]
Negative blood culture/lack of identification		20

[a] *Staph. epidermidis, Staph. hominis* (1 case); *Staph. epidermidis, Str.* sp. (1); *Staph. epidermidis, E. faecalis* (1); *Str. haemolyticus, E. coli* (1); *E. faecalis, E. coli* (1); *Staph. epidermidis, Ps. aeruginosa* (ESBL +) (1); *Staph. epidermidis; Staph. chromogenes; E. coli* (1).

[b] *Staph. epidermidis, Staph. haemolyticus* (1 case); *Staph. epidermidis, Staph. hominis* (1); *Staph. epidermidis, S. maltophila* (1); *Staph. epidermidis, Staph. haemolyticus, S. maltophila* (1); *Staph. epidermidis, Staph. haemolyticus, Staph. warneri* (1); *Staph. epidermidis; Staph. warneri, Enterobacter cloaceae* (1); *Neisseria meningitidis, Acinetobacter baumanii* (2).

[c] MR—methicilin resistant; ESBL+—extended-spectrum beta-lactamases.

The neonatal reference groups consisted of healthy controls (newborns with no infection before leaving hospital (C1: $n = 273$; mean gestational age 38.6, range 28–43)) and disease controls (DC) consisting of babies with infections (mainly pneumonias) who did not develop sepsis ($n = 40$; mean gestational age 36 weeks, range 25–40). The C1 group was composed of 48 subjects born between 2009 and 2013 and 225 randomly selected babies from the pre-2009 cohort previously described (Swierzko et al., 2009a,b,c; Michalski et al., 2012; Kilpatrick et al., 2013). Clinical material