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Opsonic Activity of Cord Blood Sera against Various Species of Microorganism

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ABSTRACT. The results of the present study on the opsonic activity of cord blood serum against various microorganisms (*Staphylococcus aureus*, *Escherichia coli*, and group B streptococci) show that the activity of cord blood serum in promoting IgG-mediated ingestion is equal to that of sera of healthy adults. This implies that IgG concentrations, as assessed by immunochemical methods, in cord blood and adult sera represent functionally similar IgG activities. Ingestion of microorganisms involving complement-dependent opsonization was found to be of the same level for cord blood and adult sera, when the opsonization occurred via the classical pathway of complement activation. However, due to decreased concentrations of factors B, P, and D in cord blood serum, optimal opsonization of microorganisms requiring the alternative pathway of complement was impaired. Taken together, these results indicate that an opsonic defect of cord blood serum affects mainly microorganisms requiring opsonization via the alternative pathway of complement. (*Pediatr Res* 19: 433–436, 1985)

Abbreviations

HBSS, Hanks' balanced salt solution
AP50, alternate pathway of complement
CH50, classical pathway of complement

Interaction between serum proteins and microorganisms may lead to the generation of chemotactic factors and may promote ingestion of microorganisms by granulocytes and monocytes (1–4). An impairment of the functional activity of human serum caused by an antibody deficiency or a complement disorder was found to be associated with recurrent and severe infections (5–8).

Investigations on the functional activity of sera of newborns as a source of opsonins showed that these sera have less opsonic activity than sera from adults. Despite this general conclusion, the published data are difficult to interpret and often contradictory (9–17). The main reason for these discrepancies lies in the use of different assays, different types of particles, particle size, and different serum concentrations. Concentrations of complement components important for the opsonization of various microorganisms in newborns were found to be relatively low compared with the levels in adults; however, normal and even elevated levels of complement proteins have also been reported (18–27).

Herein we report studies on the opsonic activity of cord-blood sera for *Staphylococcus aureus*, *Escherichia coli*, and group B streptococci as well as the concentrations of immunoglobulins and complement components in these sera.

MATERIALS AND METHODS

Sera. Five to 10 ml cord blood were collected under aseptic conditions from the placental end of the umbilical cord of 30 healthy, full-term neonates at delivery in the Department of Obstetrics and Gynaecology of the Leiden University Hospital and the Department of Gynaecology of the Diaconessenhuis in Leiden. For the control studies use was made of blood from 40 healthy donors. Serum was prepared by clotting the blood for 1 h at room temperature followed by centrifugation for 20 min at 1200 × *g*, and was stored in aliquots at −70° C. Serum from cord blood of newborns will be called cord blood serum here and serum prepared from the blood of adults will be called normal adult serum. Heat-inactivated serum was prepared by heating serum for 30 min at 56° C.

Determination of immunoglobulin concentrations. Concentrations of immunoglobulins (IgG, IgM, IgA) were determined in cord blood sera by immunodiffusion with anti-IgG, anti-IgM, and anti-IgA sera (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam), and expressed as international units. Concentrations of IgG subclasses were determined with sheep anti-human IgG subclass sera (Nordic, Tilburg, The Netherlands), according to Ouchterlony (28) and expressed as the maximal serum dilution giving a positive precipitation line; IgG subclass concentrations were compared with corresponding concentrations in a normal adult donor serum pool.

Determination of complement activities. The activity of the CH50 and AP50 activation was determined by hemolytic titration (CH50 and AP50, respectively), as described elsewhere (29, 30), and expressed in U/ml. Components C3, C4, and B were determined as described elsewhere and expressed as μg/ml (30). Properdin (factor P) levels were determined by rocket electrophoresis with a rabbit antiserum specific for factor P; concentrations are expressed as μg/ml. Factor D was determined by functional titration as described elsewhere (30) and expressed as U/ml.

Granulocytes. Granulocytes were isolated from the blood of healthy adult donors by dextran sedimentation of the erythrocytes, as described elsewhere (31), and suspended in HBSS with 0.1% gelatin to a final concentration of 10^7 cells/ml.

Microorganisms. S. aureus (type 42D), *E. coli* (054), and a

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clinical isolate of group B streptococci type III were cultured overnight at 37°C in Nutrient Broth (Oxoid Ltd, London, England), harvested by centrifugation at 1500 × *g* for 10 min, washed twice with phosphate buffered saline, and resuspended in gelatin to a concentration of approximately 10^7 bacteria/ml. All bacteria were serum resistant, *i.e.* incubation of 5 × 10^6 bacteria/ml in 10 or 90% normal serum for 2 h at 37°C did not lead to detectable decrease in the number of viable bacteria.

Preopsonization of bacteria was performed by incubating 5 × 10^6 bacteria/ml with various concentrations of normal serum for 30 min at 37° C under slow rotation (4 rpm); next the suspension was cooled to 4° C, and the excess serum removed by centrifugation and two washes with ice-cold HBSS. The bacteria were then resuspended in gelatin-HBSS to a concentration of 10^7/ml.

Determination of the opsonic activity of serum. The opsonic activity of a serum was determined as its capacity to promote ingestion of bacteria. This was measured by incubation of 100 μl of a suspension of granulocytes (concentration: 10^7 cells/ml) with an equal volume of a suspension of 10^7 bacteria/ml in the presence of various concentrations serum at 37° C and 4 rpm. At 0, 30, and 60 min, 50-μl aliquots of the mixture were added to 450 μl ice-cold HBSS, after which the cells were pelleted by differential centrifugation for 6 min at 75 × *g* and the number of the viable extracellular bacteria determined by a microbiological assay (32). In control experiments 200 μl of a suspension of 5 × 10^6 bacteria/ml were incubated under rotation (4 rpm) at 37° C with various concentrations (v/v) of the serum under study, and at various time-points the number of viable bacteria was determined microbiologically.

Since noningested bacteria proliferate during incubation of a suspension of granulocytes and bacteria, the number of viable extracellular bacteria must be corrected for this extracellular growth according to the formula (33): $NC_t = N_t \times B_0/B_t$, in which NC_t is the corrected number of extracellular bacteria at time t, N_t the number of extracellular bacteria counted at time t, B_0 the initial number of bacteria in the suspension of only bacteria and serum, and B_t the number of viable bacteria in this suspension at time t. After this correction, phagocytosis can be expressed as the percentage decrease in the corrected number of viable extracellular bacteria according to the formula:

$$P(t) = [(1 - NC_t)/N_0] \times 100$$

in which P(t) is the percentage phagocytosis at time t, and N_0 the number of viable extracellular bacteria at time t = 0.

Data are expressed as mean and SD of at least three independent experiments. Statistical analysis was performed with Student's two-tailed *t* test for unpaired observations.

RESULTS

Opsonic activity of cord blood serum. Incubation of 5 × 10^6/ml granulocytes and 5 × 10^6/ml *S. aureus*, *E. coli*, or group B streptococci in the presence of various concentrations of either cord blood or adult serum at 37° C for 60 min showed that phagocytosis of all of these microorganisms is dependent on the concentration of the serum in the medium employed (Figs. 1–3). Ingestion of *S. aureus* proved to be similar in the presence of corresponding concentrations of cord blood and adult sera (Fig. 1). Ingestion of *E. coli* was significantly reduced in the presence of all concentrations of cord blood serum as compared with adult serum (Fig. 2). For group B streptococci a slightly higher degree of ingestion was found in the presence of adult serum compared with cord blood serum (Fig. 3). Comparable results were obtained when the phagocytosis assay was performed for 120 min instead of 60 min (data not shown).

Similar results were obtained in phagocytosis experiments performed with bacteria preopsonized with various concentrations of cord blood or adult serum instead of the serum present during the phagocytosis assay (data not shown).

Opsonic activity of inactivated cord blood serum. To find out whether ingestion of microorganisms in the presence of cord blood serum is mediated mainly by heat-stable opsonins (IgG) or by heat-labile factors (complement components), the opsonic activity of heat-inactivated cord blood serum (*i.e.* IgG-mediated ingestion) was determined. Incubation of 5 × 10^6 granulocytes/ml and 5 × 10^6 *S. aureus*/ml together with heat-inactivated cord blood serum in various concentrations induced ingestion to the same degree as did corresponding concentrations of heat-inactivated adult serum (Fig. 1). Similar incubation of granulocytes and *E. coli* resulted in almost complete abolition of ingestion of these bacteria in the presence of heat-inactivated serum, whether cord or adult serum was used (Fig. 2). For group B streptococci, phagocytosis in the presence of heat-inactivated cord blood serum was similar to that in the presence of heat-inactivated adult serum when the serum concentration amounted to 1.0 or more (Fig. 3).

Immunoglobulin and complement concentrations in cord blood serum. To find out whether the opsonic activity of cord blood serum is correlated with the concentration of the various opsonic proteins, *i.e.* immunoglobulins and complement components,

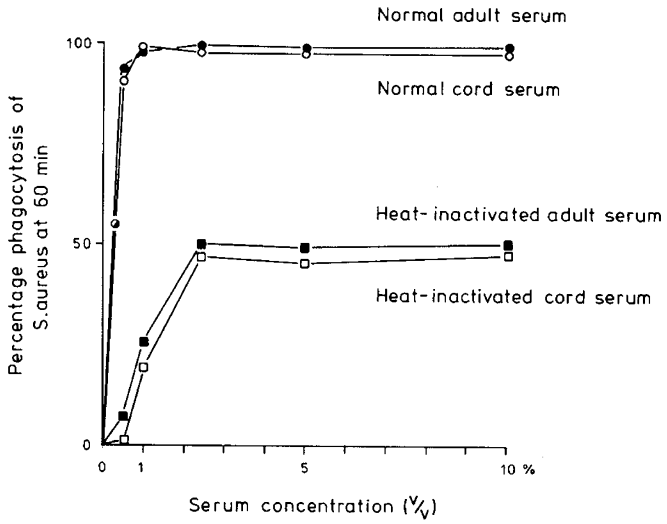

Fig. 1. Phagocytosis of *S. aureus* by granulocytes of healthy donors in the presence of the indicated concentrations of normal and heat-inactivated cord and adult sera.

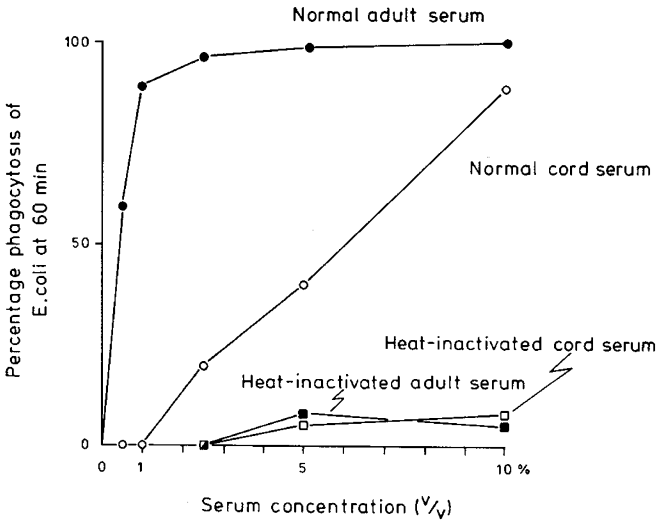

Fig. 2. Phagocytosis of *E. coli* by granulocytes of healthy donors in the presence of the indicated concentrations of normal and heat-inactivated cord and adult sera.

the level of these proteins was determined in cord blood serum. The results show no detectable IgA in cord blood serum and an absent or lower concentration of IgM in cord blood than in adult serum (Table 1). No substantial differences were found between concentrations of IgG and IgG subclasses in cord blood and adult sera (Table 1).

Determination of the levels of complement components C3 and C4 and of factors B, P, and D in cord blood serum showed slightly lower concentrations of all five compared with those found in adult sera (Table 2). Investigation of complement activities of cord blood serum showed that although the capacity to activate the CH50 differed between the various sera, the mean CH50 activity was only slightly lower in cord than in adult sera. In all but one of the cord sera the level of at least one factor of the alternative pathway (B, D, or P) was lower than in sera from adults, which explains why the ability to activate the AP50 was almost negligible in these sera (Table 2).

Complement activation by various microorganisms. To assess the capacity of the above mentioned microorganisms to activate the CH50 and AP50, 5×10^7 bacteria were incubated with 50% normal adult serum for 30 min at 37° C. The suspension was then cooled to 4°C, the bacteria removed by centrifugation at $1500 \times g$ for 10 min, and the residual complement activity in the supernatant determined. The results showed a total loss of the classical pathway activity by *S. aureus*, *E. coli*, and group B streptococci (Table 3). With respect to the alternative pathway *S. aureus* induced 22% consumption of AP50 activity, while group B streptococci did not induce any consumption (Table 3). *E. coli* induced 100% consumption of AP50 activity (Table 3).

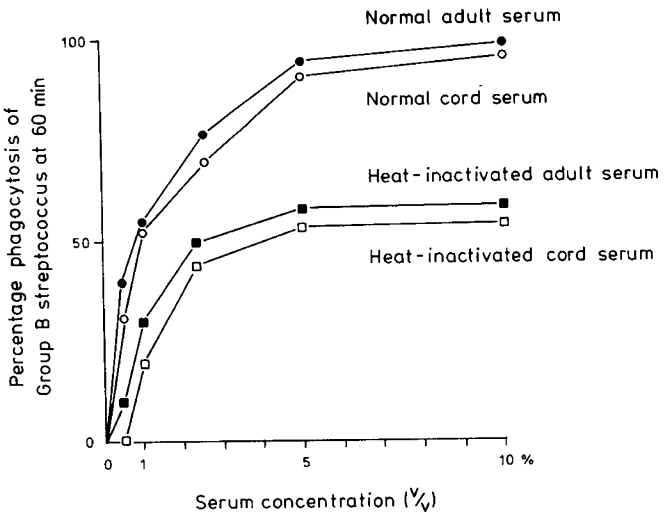

Fig. 3. Phagocytosis of group B streptococci by granulocytes of healthy donors in the presence of the indicated concentrations of normal and heat-inactivated cord and adult sera.

DISCUSSION

In the present study cord blood serum proved to be as active as serum from healthy adults for the opsonization of microorganisms by IgG and complement when the latter is activated via the CH50, which is the case for *S. aureus* and group B streptococci. Cord blood serum is inadequate for optimal opsonization when microorganisms, *e.g. E. coli*, activate the AP50 as well. The absence of activation of the AP50 by cord blood serum is in all probability due to a decreased level of at least one of the factors, B, P, and D of the AP50 in cord blood serum (24, 26).

Ingestion of *S. aureus* and group B streptococci in the presence of heat-inactivated adult serum and heat-inactivated cord blood

Table 3. *Complement activation by various species of microorganism**

Microorganism	Residual CH50 (%)	Residual AP50 (%)
S. aureus	0	78
E. coli	0	0
Group B streptococci	0	100

* Determinated after incubation of 5×10^7 microorganisms with 50% donor AB serum for 30 min at 37° C.

Table 1. *Levels of immunoglobulins in cord blood and adult sera*

Source of Serum	Immunoglobulins						
	IgA*	IgM*	IgG*	IgG_1†	IgG_2†	IgG_3†	IgG_4†
Cord blood							
Mean	0	4	110				
Median	0	3	108	1/250	1/64	1/64	1/8
Range	0	0–11	84–145	1/64–1/500	1/16–1/250	1/16–1/64	1/2–1/32
Adult blood							
Range	50–270	60–250	80–220	1/250	1/64	1/64	1/8

* Expressed as IU/ml.

† Expressed as maximal dilution giving precipitation.

Table 2. *Complement activity and levels of complement components in cord blood and adult sera*

Source of serum	Complement activity		Complement				
	CH50 (U/ml)	AP50 (U/ml)	C3 (μg/ml)	C4 (μg/ml)	B (μg/ml)	P (μg/ml)	D (U/ml)
Cord blood							
Mean	183	2	700	170	80	14.7	43
Median	191	2	710	160	80	14.3	33
Range	0–374	0–6	490–830	50–400	40–180	0–19	0–100
Adult blood							
Range	256–580	8–24	690–1040	170–300	130–220	17–18	25–105

serum indicates that in cord blood serum IgG, which is present in the same concentration as in adult serum, is functionally active. The higher level of ingestion of *S. aureus* and group B streptococci in the presence of cord blood and normal adult sera than in the presence of heat-inactivated sera indicates that opsonization is optimal when complement can be activated to opsonize these bacteria. Both of these species of bacteria can activate the classical pathway of complement only; the AP50 slightly or not at all. The absence of ingestion of *E. coli* in the presence of heat-inactivated cord blood and adult serum indicates that the ingestion of *E. coli* is mainly complement mediated. The decreased ingestion of *E. coli* in less than 10% cord blood serum compared to adult serum indicates that, although *E. coli* is able to activate both pathways of complement, ingestion of *E. coli* is mainly mediated by opsonization via the AP50.

IgM antibodies can play a role in the opsonization of *E. coli* by activating complement via the classical pathway, but cord blood serum does not contain IgM, because this immunoglobulin does not cross the placenta. Addition of IgM to cord blood serum did not suffice to obtain maximal phagocytosis of *E. coli in vitro* (11, 34).

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