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Complement properdin newborn

Deficient Alternative Complement Pathway Activity in Newborn Sera

ELAINE L. MILLS, BENGT BJÖRKSTEŃ, PAUL G. QUIE

Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota, USA, and Department of Pediatrics, University of Umeå, Umeå, Sweden

Summary

Neonatal susceptibility to overwhelming bacterial infection is commonly attributed to a relative deficiency in serum opsonic activity. However, few studies have compared the functional capacity of the classical complement pathway with that of the alternative complement pathway in the neonate. The opsonic activity of nine maternal infant serum pairs were studied by determining percent uptake of radiolabeled *Escherichia coli.* Seven mother-infant paired sera were studied using *E. coli* strains known to be opsonized via the alternative complement pathway: the mean percent uptake of *E. coli* opsonized in neonatal sera was 16.8%; of those opsonized in maternal sera, 54%; and of those opsonized in control sera, 45% ($P < 0.005$). Two *E. coli* strains requiring the classical complement pathway for opsonization were phagocytized equally well in maternal and infant sera of seven mother-infant pairs. Determination of anti-O hemagglutination inhibition (HI) antibody titers in six maternal sera for one classical complement pathway activating and one alternative complement pathway strain showed no correlation between percent phagocytosis and HI antibody titer.

These data would suggest that serum levels of classical pathway components are probably adequate for opsonization of *E. coli* via the classical pathway, but that low alternative complement pathway activity in neonatal sera may contribute to the newborn's increased susceptibility to bacterial sepsis.

Speculation

There is good evidence that both humoral and cellular deficiencies exist in the inflammatory response of the newborn. The pathogenesis of neonatal *E. coli* sepsis and meningitis may involve a critical balance between the presence of these immunologic deficiencies and the particular opsonic requirements of the invading organisms. In the adult host, many strains of *E. coli* are well opsonized by either specific antibody or by activation of the alternative complement pathway. In the neonate, in the absence of transplacentally derived specific antibody, efficient opsonization of these same organisms cannot be accomplished due to low levels of alternative complement pathway activity. Thus, treatment rationale in *E. coli* sepsis and meningitis may include supplementation with either or both specific antibody and a source of complement activity.

Neonatal susceptibility to overwhelming bacterial infection is commonly attributed to a relative deficiency in serum opsonic activity. Decreased opsonic activity in neonatal sera compared to that of adults has been reported for a variety of test particles (4, 7, 10, 11, 15–19). Moreover, several opsonic factors are decreased in neonatal serum compared to that of adults, *i.e.*, all components of the classical complement pathway and C3 proactivator and properdin of the alternative complement pathway, as well as immunoglobulin IgM and IgA (5, 6, 9, 13). Few studies have compared the functional capacity of the classical complement pathway with that of the alternative complement pathway in these sera. One study found that whereas most neonatal sera had decreased opsonic activity for *E. coli*, both neonatal and maternal sera required the presence of maternally derived specific IgM antibody as well as complement for efficient opsonization (4). The decreased activity of neonatal sera was attributed to a relative deficiency of IgM opsonic antibody. Another study showed that 6 of 40 cord sera with very low opsonic activity for *E. coli* also had very low levels of C3 proactivator (18).

The fact that previous studies have not been consistent with regard to defective opsonic capacity for *E. coli*, may be related to our recent observation that *E. coli* strains vary in their opsonic requirements. Both qualitative and quantitative variation in the opsonic requirements of different *E. coli* strains have been found (2). Therefore, in the present study, the ability of neonatal sera to opsonize various strains of *E. coli* with known opsonic requirements were compared. The functional activity of classical pathway and alternative pathway opsonization in newborn and maternal sera were determined by measuring polymorphonuclear neutrophil (PMN) uptake of radiolabeled *E. coli.*

MATERIALS AND METHODS

OPSONINS

The study population consisted of 11 healthy newborn-mother pairs. Control serum was obtained from normal adult female donors. Blood was drawn from the newborn infants and their mother 12–36 hr after delivery. Infants were 38–42 wk of gestation, of appropriate size for gestational age, free of congenital anomalies, stress, or trauma and had Apgar scores of at least 8 at 5 min. All had at least one feeding of glucose, bottle, or breast milk. Mothers were normal by history and physical examination with uncomplicated pregnancies, labor,and delivery. Amniotic membranes were ruptured for no more than 12 hr.

Sera obtained from infants, mothers, and controls were frozen in aliquots at −70° C within 1 hr of blood drawing. Shortly before use, the aliquots were thawed and diluted to a final concentration of 10% in Hank's balanced salt solution HBSS.

BACTERIA

Serum resistant strains of *E. coli* serotypes O22:H16, O18:K1:H7 (2 strains) and O15:K7:H- were used. *E. coli* O22:H16 was a strain isolated from the stool of a healthy newborn whereas the other three strains were obtained from the cerebrospinal fluid of sick infants. All four strains have previously been reported (2). Strains O22:H16 and O15:K7:H- were well opsonized in 2% pooled human serum chelated with 10mM MgEGTA *i.e.*, opsonized via alternative complement pathway (8). The two strains of serotype O18:K1:H7 required classical pathway of complement activation for opsonization because they were not opsonized in MgEGTA chelated serum.

LEUKOCYTES

Leukocytes were obtained for the opsonic assay from the heparinized venous blood (10 U heparin/ml) of healthy donors. Erythroyctes were sedimented for 1 hr in 6% dextran (Cutter laboratories, Berkeley, CA). The leukocyte pellet was washed twice in heparinized saline (10 U heparin/ml). After total and differential counts, the leukocyte pellet was resuspended in Hank's balanced salt solution in 0.1% gelatin to give a final concentration of 1×10^7 PMN/ml.

PHAGOCYTOSIS ASSAY USING RADIOACTIVE LABELED BACTERIA

The methods employed for radioactive labeling, bacterial opsonization, phagocytosis mixtures, and determination of PMN uptake are those previously described in detail (21). Briefly, mixtures of leukocyte suspension, opsonin, and bacteria were tumbled at 10 rpm in a rotating rack at 37° C. Duplicate 100 ul samples were drawn from the test mixtures at 1, 10, and 20 min, washed three times in phosphate buffered saline (PBS) and resuspended in scintillation liquid. The percent of the total bacterial population that was leukocyte associated at a given sampling time (% uptake) was calculated using the formula:

$$\% \text{ uptake} = \frac{\text{counts per minute in leukocyte pellet}}{\text{total counts per minute}} \times 100.$$

ANTIBODY DETERMINATIONS

Hemagglutination inhibiting antibody directed at the somatic O-antigen of the *E. coli* strains was measured in the maternal sera by the method described by Anderson (1). To evaluate IgM antibody titers for *E. coli*, the sera were treated with mercaptoethanol as described by Holmgren (12).

RESULTS

ALTERNATIVE COMPLEMENT PATHWAY OPSONIC ACTIVITY

Sera from six newborns, their mothers, and healthy adult controls were studied for alternative pathway opsonic activity using *E. coli* strain O22:H16, a strain which had previously been shown to be opsonized effectively via the alternative complement pathway (2). Figure 1 show the uptake of *E. coli* by PMN after opsonization of bacteria in the various sera. Each mother-infant serum pair and a control serum were assayed simultaneously. The *E. coli* incubated in neonatal sera were poorly opsonized and there was little phagocytosis (mean 16.8%) whereas there was good uptake in maternal serum (mean 54%) and in control sera (mean 45%) ($P < 0.005$) Table 1. A similar difference in opsonic activity between neonatal, maternal and control sera was found when another strain of *E. coli* (O15:K7:H) (also effectively opsonized via the alternative complement pathway) was studied with an additonal mother-infant paired sera (data not shown).

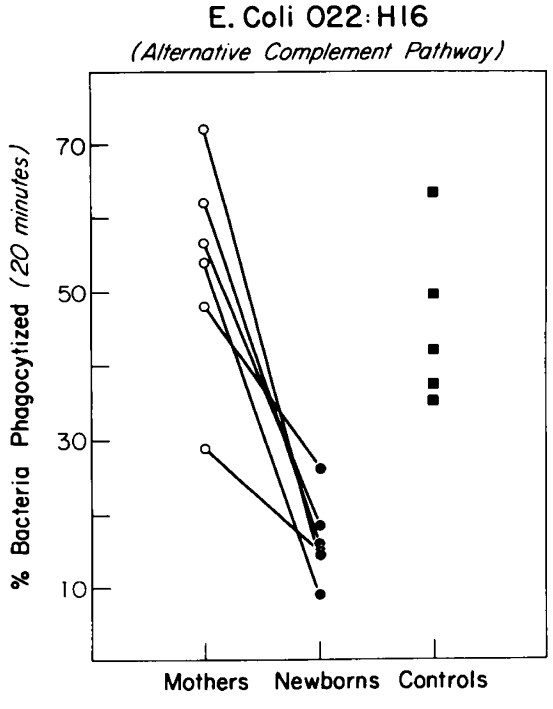

Fig. 1. Phagocytosis of radiolabeled bacteria opsonized in maternal, newborn and control sera. Phagocytosis mixtures consisted of 5×10^6 control PMN and 5×10^7 radiolabeled bacteria preopsonized in 10% test sera. *E. Coli* O22:H16 has previously been shown to be well opsonized (>90% uptake of radiolabeled bacteria) in 2% pooled human serum chelated with 10 mM MgEGTA indicating opsonization occurred via activation of the alternative complement pathway.

Table 1.

Maternal serum	*E. coli* 022:H16 (alternative complement pathway)		*E. coli* 018:K1:H7 (classical complement pathway)	
	HI titer[1]	Percent phagocytosis	HI titer	Percent phagocytosis
1	1:8	29	1:8	
2	1:16	48	1:8	64
3	1:16	72	1:16	60
4	1:32	54	1:16	53
5	1:32	57	1:16	71
6	1:32	64	1:32	37

[1] Hemagglutination inhibiting antibody titer for 0 antigen.

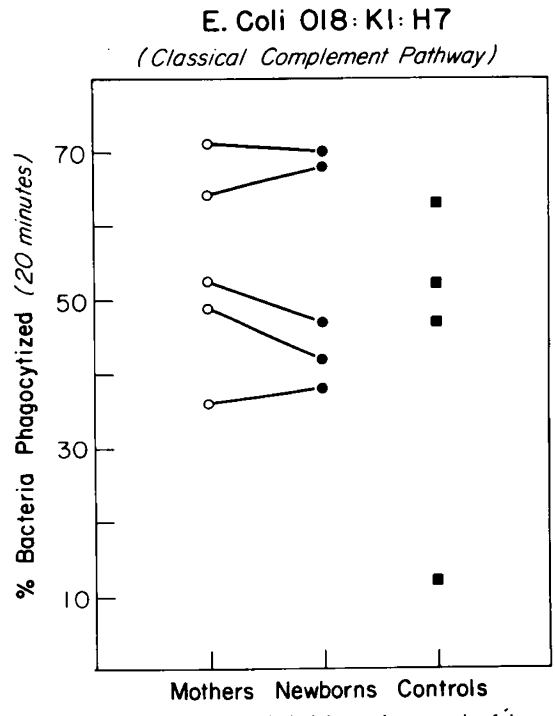

Fig. 2. Phagocytosis of radiolabeled bacteria opsonized in maternal, newborn and control sera. Phagocytosis mixtures were as described for Figure 1. *E. Coli* O18:K1:H7 has previously been shown to be poorly opsonized in 10% pooled human serum chelated with 10 mM MgEGTA indicating opsonization requires activation of the classical complement pathway.

CLASSICAL COMPLEMENT PATHWAY OPSONIC ACTIVITY

Sera from five of these same six mother-infant serum pairs were studied for classic pathway opsonic activity using *E. Coli* strain 018:K1:H7 that had previously been shown requires the classic complement pathway for opsonization (2). Figure 2 shows the uptake of *E. coli* by PMN after opsonization of bacteria in the various sera. Each mother-infant serum pair and control were assayed simultaneously. This *E. coli* strain was opsonized equally well in newborn, maternal, and control sera. The mean uptake of bacteria opsonized in neonatal sera was 53%, in maternal sera 56%, and in control sera 44%. Another strain of *E. coli* (018:K1:H7) was studied with two additional mother-infant serum pairs and results confirmed those shown in Figure 2 (data not shown).

DETERMINATION OF ANTI-O ANTIBODY TITERS

The titer of HI antibody directed against the O antigen of *E. coli* strains O22:H16 and O18:K1:H7 were determined in the six maternal sera (Table 1). All sera contained antibodies and the titers ranged from 18–164. There was no correlation between titer of antibody and opsonic activity of individual sera. When these sera were treated with mercaptoethanol, all hemagglutination inhibition activity was destroyed, suggesting that the antibody activity for the O antigen in maternal sera was primarily IgM. The antibody titers of the infant sera were not determined.

DISCUSSION

The authors found that serum from all of eight healthy term newborns had markedly reduced opsonic activity when tested using two strains of *E. coli* (O22:H16 and O15:K7:H) known to be effectively opsonized via the alternative complement pathway. In contrast, seven infant sera had normal opsonic activity when tested using two strains of *E. coli* (O18:K1:H7) known to be effectively opsonized via the classical complement pathway. Opsonic activity in the neonatal sera was comparable to that paired of maternal and control sera. This study confirms the report of Stossel *et al.* (18) that alternative complement pathway opsonic function may be defective in newborn infants.

Previous studies have shown reduced opsonic activity in cord and neonatal sera to a wide variety of agents (4, 7, 10, 11, 15–19). However, direct comparison of the results from these investigations to the present findings are not possible due to variability in type, specificity, and sensitivity of the *in vitro* assays employed. Evaluation of opsonic activity in neonatal serum has been hampered by the lack of methodology specific for evaluating opsonization as a discrete step in phagocytosis, *i.e.*, one separable from attachment, ingestion, and killing. The present study utilized an assay employing radiolabeled organisms in which simultaneous, separate, and quantitative assessment of each of these processes is possible. The sensitivity of the method allows detection of subtle differences in opsonic activity and is highly reproducible (21, 20).

It has only recently been appreciated that different strains of *E. coli* have markedly different opsonic requirements. Certain strains are effectively opsonized via the alternative pathway whereas others require classical complement activation (2). *E. coli* cell wall lipopolysaccharides have been shown to directly activate complement via the classical pathway in the absence of antibody (14). Therefore, this or a similar cellular constituent may contribute to the variable opsonic requirements of different *E. coli* strains. The *E. coli* O22:H16 strain was chosen because it completely decomplemented sera in the absence of specific antibody (absorbed sera) and under conditions in which the classic pathway was blocked by MgEGTA chelation. This strain was efficiently opsonized in as little as 2% MgEGTA chelated adult serum. The poor opsonic activity of neonatal sera, therefore, appears to be a result of defective alternative complement pathway activation. The normal opsonic activity of neonatal sera for *E. coli* strain O18:K1:H7 is evidence that classical complement activation was intact in these newborn sera. Neonatal and maternal sera in each mother-infant pair had comparable opsonic capacity for these organisms, suggesting the presence of transplacentally transferred factors. The nature of these opsonic factors was not studied.

The role of the K1 antibody in the opsonization of K1 positive *E. coli* has not been established. Few studies have correlated K1 antibody titers with opsonic activity. Welch *et al.* (22) have recently shown that K1 positive antisera was essential for the opsonization of an O7:K1 *E. coli* strain which was resistant to phagocytosis in control sera. In addition, they showed absence of opsonic activity in antisera directed to the core glycolipid, lipid A, or the O7 somatic antigen. Whether these opsonic requirements extend to other *E. coli* strains has not be demonstrated. Regardless of the type of opsonic antibody required by these strains, previous studies would indicate that it is not uncommon for adult sera to lack opsonic activity for a number of K1 positive *E. coli* strains (2, 3). This apparently was not the case in these studies, however, because the paired maternal infant sera had comparable opsonic activity for *E. coli* strains opsonized via the classical pathway.

The clinical relevance of our finding that neonatal sera had very low alternate complement pathway activity is at present unknown. The majority (54–84%) of *E. coli* strains associated with meningitis in the newborn contain the K1 capsular polysaccharide antigen (2, 23) and are opsonized via the classical complement pathway (2, 24, 25). Most of these *E. coli* K1 strains also appear to be more virulent for neonatal rats (26) and more resistant to phagocytosis by normal neutrophils (24, 26, 27) than non-K1 strains. Moreover, a protective role for anti-K1 capsular polysaccharide antibody has been demonstrated by the finding that anti-K1 capsular polysaccharide antibodies, passively administered to neonatal rats or induced by active immunization of pregnant rats, confer immunity toward invasive disease caused by *E. coli* K1 in these neonates (28). The majority of newborns have been reported to have IgG K1 antibody in cord sera in titers comparable to maternal titers (29). The infants at greatest risk for K1 *E. coli* sepsis then would appear to be those infants who lacked this antibody. The contribution of alternative complement pathway activity to efficient *E. coli* opsonization in the presence of antibody is not known and may be of importance.

A significant number of *E. coli* strains (16–46%) (2, 25) associated with neonatal meningitis do not contain K1 antigen and do not require classical complement pathway activity for opsonization. The great majority of these strains are efficiently opsonized in the alternative complement pathway (2).

Newborn infants, therefore, without specific antibody and with defective alternative pathway activity may be in double jeopardy for *E. coli* sepsis,. The acquisition of cross reacting *E. coli* antibodies and the maturation of alternative complement pathway activity may be associated with the relative immunity to *E. coli* sepsis observed in older infants.

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33. Requests for reprints should be addressed to: Elaine Mills, M. D., Department of Pediatrics, University of Minnesota Medical School, Box 483, Minneapolis, MN 55455 (USA).
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