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Murine Hybridoma Antibodies Enhance Bactericidal Activity of Human Cord Blood against K1 *Escherichia coli* Strains[1]

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ABSTRACT. Murine hybridoma antibodies directed against the capsule and O-side chain determinants of the *Escherichia coli* strain Bort (018ac:K1:H7) were evaluated for their ability to enhance bactericidal activity of cord blood against K1 *E. coli* strains possessing O antigens common in neonatal *E. coli* infection, *i.e.* 018, 07, and 01. The antibodies to the K1 capsule and O-side chain efficiently enhanced cord polymorphonuclear leukocyte-mediated killing of K1 encapsulated *E. coli* strain possessing a homologous O antigen, but the IgM antibody to the K1 capsule exhibited approximately 10-fold greater activity than did the IgG3 antibody to O-side chain (weight basis). Both antibodies required complement for their opsonic activities. Our findings indicate that antibodies directed against the capsule and O-lipopolysaccharides are able to restore the opsonic activity of cord blood against K1 *E. coli*, suggesting that these antibodies may be useful in the prevention and therapy of neonatal *E. coli* infection. (*Pediatr Res* 28: 667–670, 1990)

Abbreviations

CFU, colony forming units
LPS, lipopolysaccharide
PMN, polymorphonuclear leukocyte

In neonates, infections due to gram-negative bacilli are associated with significant morbidity and mortality despite appropriate antimicrobial therapy (1). The most common gram-negative organism causing sepsis and meningitis during the neonatal period is *Escherichia coli* (1). Given the plethora of *E. coli* serotypes (2), it is striking that *E. coli* strains possessing the K1 capsular polysaccharide are the predominant capsular serotype responsible for neonatal *E. coli* sepsis and meningitis (3–5) and that most of these K1 *E. coli* isolates are associated with a limited number of O-LPS antigens (*i.e.* 018, 07, 01, 016) (6).

We have developed a series of murine hybridomas producing MAb against different cell wall components of K1-encapsulated *E. coli*. MAb prepared against group B meningococcus and reactive with *E. coli* K1 polysaccharides and antibodies directed against O-side chain determinants of a K1 *E. coli* strain were opsonic *in vitro*, and also protected newborn rats against a homologous O serogroup of K1 encapsulated *E. coli* (7). The previous opsonic experiments used adult human PMN and fresh adult serum against K1-encapsulated *E. coli* strains possessing a homologous O serogroup. Our study evaluated whether these MAb could also provide opsonic activity of cord blood against K1 *E. coli* strains.

MATERIALS AND METHODS

Bacterial strains. Three serum-resistant K1-encapsulated *E. coli* strains were tested; C5 (018ac:K1:H7), C10 (07:K1:NM), and A90 (01:K1:H7). These strains were isolated from the cerebrospinal fluid of newborn infants with meningitis and were kindly provided by R. Bortolussi of Dalhousie University, Halifax, Canada and M. Achtman of Max-Planck-Institute, Berlin, Germany (8, 9). The *E. coli* strains were grown in brain heart infusion broth (Difco Laboratories, Detroit, MI) to late logarithmic phase and were stored in aliquots at −70°C until used.

Hybridoma antibodies. Two murine MAb were evaluated. Clone 19 was prepared against the whole organism of *E. coli* strain Bort (018ac:K1:H7), and secreted antibody of IgG3 class (10). This MAb reacts with the O-side chain of the LPS of Bort strain and does not react with LPS core determinants (11). Quantitation of antibody in ascitic fluid by a solid-phase RIA (12) revealed the presence of 1.4 mg/mL of IgG antibody. Clone 2-2-B was prepared against group B meningococcus and secreted IgM antibody specific for the capsular polysaccharides of group B meningococcus and K1 *E. coli* (13). The ascitic fluid used in our experiment contained 3 mg of the antibody/mL as determined by a quantitative solid-phase RIA (12). For the purpose of the manuscript, this antibody is referred to as anti-*E. coli* K1 polysaccharide.

Human cord sera. Human cord sera were collected aseptically from deliveries of healthy, term infants, and only those that were free of maternal blood contamination (defined as IgM <15 mg/dL) were included in this study. Cord blood was allowed to clot at 0°C (in ice) and serum was stored in aliquots at −70°C within 60 min of collection to preserve endogenous complement activity. At the time of *in vitro* studies, sera were examined for complement activity using a CH 100 assay (Kallestad Laboratories, Austin, TX).

Opsonophagocytic assays. A total of 28 cord sera that met the above criteria were examined for the opsonic activity against three different strains of K1 *E. coli* by the method described previously (7). Neutrophils were isolated from cord blood by dextran sedimentation followed by Ficoll-Hypaque density centrifugation. Frozen bacteria were thawed, diluted (1:20) in fresh prewarmed brain heart infusion broth, and grown at 37°C for approximately 4 h to match the turbidity of a MacFarland no. 0.5 standard (14). This late logarithmic culture was washed and

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resuspended in a concentration of 1×10^8 CFU/mL. Washed neutrophils (approximately 1×10^6 PMN/40 μL) were added to a 12 × 75 mm sterile polystyrene tube (Falcon Plastics, Oxnard, CA) along with 10 μL of washed logarithmic-phase *E. coli* (approximately 1×10^6 CFU), 10 μL of cord sera, 10 μL of MAb, and 30 μL of minimal essential medium with Earles' balanced salt solution (GIBCO Laboratories, Santa Clara, CA) in a total volume of 100 μL. The same minimal essential medium was used in washing and resuspending PMN and bacteria and as a diluent of antibodies. The final mixture contained a ratio of PMN to bacteria of approximately 1:1. Control tubes lacking either PMN, cord sera, or antibody were also included in each experiment. In some experiments, cord serum heated at 56°C for 1 h was included as a control. Tubes were incubated at 37°C with end-over-end rotation using a multipurpose rotator (Scientific Industries, Inc., Springfield, MA). Aliquots (10 μL) were taken from each tube at 0 and 1 h, serially diluted 10-fold in sterile distilled water, and plated on blood agar for determination of surviving CFU. Results were expressed as the percentage of bacteria killed: 100 − [(CFU at 1 h/CFU at 0 h) × 100].

Statistical methods. Fisher's exact test, nonparametric Wilcoxon signed rank test, or paired *t* test was used where indicated (15).

RESULTS

Opsonic activity of cord sera after addition of IgM antibody reactive with K1 polysaccharide. In opsonophagocytic assays, bacterial killing did not occur in control tubes lacking either PMN, cord serum, or antibody. Thus, phagocytosis and killing of serum-resistant K1 *E. coli* strains require PMN, heat-labile serum factors (presumably complement), and specific antibody. MAb 2-2-B, reactive with group B meningococcal and *E. coli* K1 polysaccharides, was highly effective in enhancing opsonic activity of cord sera and its activity was optimal at 50–500 ng/mL of the antibody. We previously showed that the activity of this MAb was specific by providing protection against *E. coli* strains possessing the K1 polysaccharide, but not against non-K1 *E. coli* (*e.g.* K92 strain, 16). Overall, enhancement of cord PMN-mediated killing appeared to be greater with strain A90 than with strain C5 or C10 (Fig. 1). With the addition of 50 ng/mL of anti-*E. coli* K1 antibody, the mean (±SE) of killing after 1 h incubation of strain A90 was 97 ± 0.2 *versus* 83 ± 4.2% killing for strain C5 and 85 ± 2.4% killing for strain C10 ($p < 0.005$ comparing A90 to the other two strains). Efficient opsonophagocytosis and killing (defined as ≥80% killing of the original inoculum at 1 h of incubation) in the presence of 50 or 500 ng/mL of the antibody was noted in 100, 78, and 86% of the 28 cord sera tested against strains A90, C5, and C10, respectively ($p > 0.1$). Demonstration of efficient opsonic activity of cord blood in the presence of 50–500 ng/mL of the antibody was not directly correlated with CH 100 values; of the 20 cord sera exhibiting ≥80% killing against three strains of K1 *E. coli*, 12 (60%) contained >70 CH 100 U/mL (normal range), whereas three (37.5%) of eight cord sera exhibiting <80% killing against one or more of the three K1 *E. coli* strains also contained >70 CH 100.

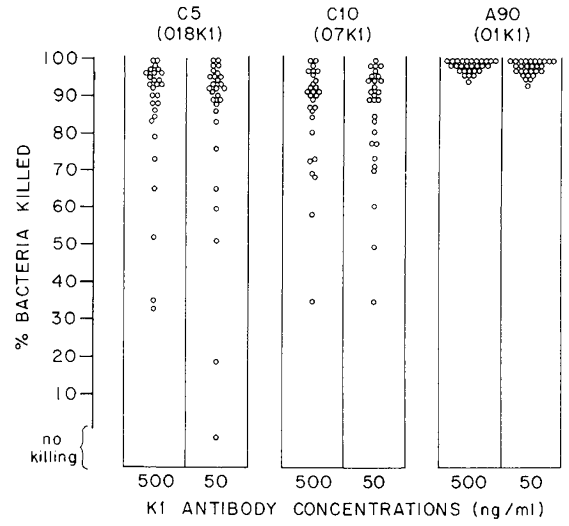

Fig. 1. Opsonophagocytic killing of serum resistant K1 *E. coli* strains C5 (018:K1), C10 (07:K1), and A90 (01:K1) by 28 cord sera in conjunction with cord PMN and IgM MAb to the K1 capsule. No killing was observed in the absence of serum, PMN, or MAb.

Opsonic activity of cord sera after addition of IgG antibody reactive with 018-LPS. We next examined the opsonophagocytic activity of cord PMN and cord sera after the addition of IgG3 anti-LPS MAb reactive with K1 *E. coli* strains possessing the 018 serotype. As expected, the opsonic activity of this anti-LPS MAb in the presence of cord sera was serotype-specific and its opsonic activity was demonstrated only against strain C5 (018:K1) and not C10 (07:K1) or A90 (01:K1) (Fig. 2). As shown previously (7), a prozone effect was observed with the LPS antibody in concentrations >5000 ng/mL. Optimal killing occurred at 500 ng/mL for all 28 cord sera tested. The mean (±SE) bacterial killings at the LPS antibody of 500 and 5000 ng/mL were, respectively, 81 ± 2.9 and 64 ± 4.9%, and this difference was significant ($p = 0.0004$). When assays were done with 500 ng/mL of the antibody, efficient opsonophagocytosis and killing (≥80% killing of the original inoculum at 1 h of incubation) occurred in 19 of 28 (68%) cord sera.

One interesting observation was that the two MAb interacted independently with some cord sera for opsonophagocytosis and killing of strain C5. For example, nine of the 28 cord sera tested failed to exhibit ≥80% killing of strain C5 in the presence of 500 ng/mL of IgG3 anti-LPS antibody. Of these nine sera, seven supported killing of ≥80% with 50 or 500 ng/mL of the IgM antibody reactive with the K1 polysaccharide. Conversely, six of the 28 cord sera failed to support ≥80% killing of strain C5 after the addition of IgM anti-K1 antibody. Of these six, four were

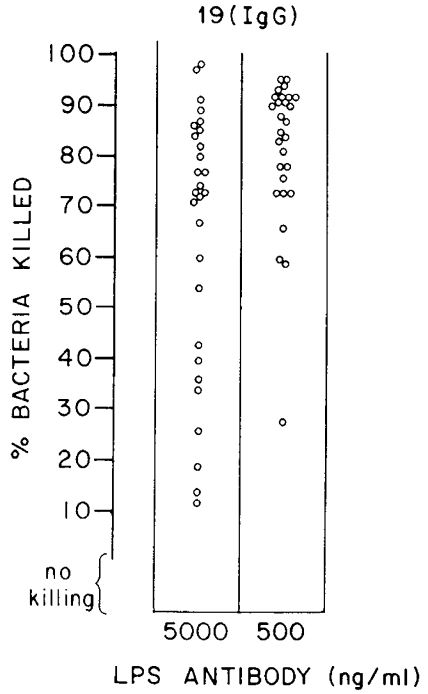

Fig. 2. Opsonophagocytic killing of a serum-resistant K1 *E. coli* strain C5 (018:K1) by 28 cord sera in conjunction with cord PMN and IgG3 MAb to a homologos serotype (018). No killing was observed in the absence of serum, PMN, or MAb, nor against K1 *E. coli* strains possessing a heterologous O serogroup (*e.g.*, 07, 01).

Table 1. *Demonstration of opsonic activity of 13 cord sera against serum-resistant K1 E. coli strain C5 (018:K1) by MAb directed against K1 capsular polysaccharide or homologous O serotype (018)*

Cord serum no.	CH 100 (U/mL)*	% Bacteria killed†	
		018	K1
15	63	59	95
20	>175	60	95
9S	55	66	91
1S	85	73	97
8S	65	73	90
22	44	74	99
6S	71	78	99
10S	95	91	35
3S	65	81	65
24	63	90	73
30	49	85	79
7	112	78	66
31	85	28	76

* >70 CH 100 U/mL is considered normal range.

† Percent of the original inoculum killed in the presence of 500 ng/mL of the IgG3 antibody to 018, or 50 or 500 ng/mL of the IgM antibody to the K1.

able to exhibit ≥80% killing with 500 ng/mL of the IgG3 anti-LPS antibody. This salutary effect on opsonic activity of cord sera by MAb directed against the capsule and O-LPS was not directly correlated with CH 100 values (Table 1).

DISCUSSION

Our studies revealed that murine IgM MAb directed against the *E. coli* K1 capsule efficiently enhanced cord serum/PMN-mediated killing of K1 *E. coli* strains regardless of their O serotypes. Further, murine IgG3 MAb to O-LPS components of K1 *E. coli* was able to support cord serum/PMN-mediated killing of *E. coli* strain possessing a homologous O serotype even in the presence of the K1 capsule. These findings corroborate those of Cross *et al.* (17) who have shown that PMN obtained from cord blood in conjunction with specific anticapsular antibody and complement can kill K1-encapsulated *E. coli* strains. Our data suggest that IgM antibody directed against the K1 capsule is more effective in providing opsonic activity against K1 *E. coli* strains than IgG3 antibody directed against O-LPS, as shown by approximately 10-fold less antibody required for efficient opsonic activity by the former than the latter. These findings are consistent with those of our previous studies using adult PMN (7). Whether this difference in complement-mediated enhancement of PMN killing reflects the different antigenic targets of the two antibodies or the different isotypes requires further investigation. Previous studies using *Haemophilus influenzae* type b indicate that IgM antibody to the type b capsule is more active on a weight basis than IgG antibody in enhancing complement-mediated bacterial lysis in the absence of PMN (18). However, IgG antibody appears to be more active than IgM antibody in enhancing PMN phagocytosis (18).

In our study, the addition of increasing amounts of IgG3 anti-O LPS antibody resulted in less bacterial killing, but a similar phenomenon was not observed at the two IgM anti-K1 antibody concentrations tested (Fig. 1). Using different opsonophagocytic assays and adult PMN (7, 13), we have previously shown that MAb directed against both the K1 capsule and O-LPS in excess of 600 μg/mL were unable to support adult PMN-mediated killing of K1 *E. coli* strain (7). The *in vivo* significance of this prozone phenomenon is not established, but our data suggesting that a prozone may occur with anti-LPS antibody concentrations as low as 5 μg/mL suggest that further studies of the mechanism and biologic relevance of this phenomenon may be warranted.

One interesting finding in our study was that although the overall ability to provide opsonic activity of cord sera was more effective with the IgM anticapsular antibody than with the IgG3 anti-O-LPS antibody, efficient opsonic activity was achieved in some sera only with the anti-O-LPS antibody (Table 1). The reasons for this apparent discrepant interaction of anti-O-LPS antibody with cord sera are unclear. We previously showed in the experimental *E. coli* infection that the combination of antibodies to the capsule and O-LPS was more beneficial than either antibody alone because it provided significantly greater protection from bacteremia and death (7). Thus, one potential explanation for the greater activity observed with the anti-O-LPS antibody in some cord sera may be that these cord sera contain suboptimal concentrations of other opsonic antibodies. Thus, although these sera did not exhibit efficient opsonic activity by themselves, endogenous antibody directed against different cell wall components (*e.g.* capsule) may have interacted with the MAb added to the reaction and resulted in efficient opsonophagocytosis and killing of a homologous K1 *E. coli.* Additional studies are needed measuring directly the presence of such antibodies or using cord sera absorbed with non-LPS structures (*e.g.* capsule) to refute this possibility. Nevertheless, our findings support the concept that IgG antibody to O-LPS may be useful for passive immunotherapy of neonatal K1 *E. coli* infection. This information is particularly valuable because the number of O antigens common in neonatal *E. coli* sepsis and meningitis are limited (6) and techniques for preparing hyperimmune globulin-containing antibodies against several O antigens are available.

Overall, our studies indicate that cord PMN and sera are deficient in exhibiting opsonic activity against K1 *E. coli* strains possessing O antigens commonly associated with neonatal sepsis and meningitis (*i.e.* 018, 07, and 01) and that antibodies directed against the capsule and O-LPS can restore the opsonic activity of cord blood against these K1 *E. coli* strains. However, use of antibody preparations to the K1 polysaccharide or efforts to improve the K1 polysaccharide's immunogenicity must be pursued with caution. Certain structures (*e.g.* gangliosides, glycopeptides) in the human fetal and newborn brain contain sialic acid with the same α-2,8-linkage as *E. coli* K1 polysaccharides (18–20). Thus, there is some concern whether antibodies to the K1 polysaccharide may bind to host tissues and have a harmful effect (21).

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