Monoclonal Antibody 2-2-B Kills K1-positive *Escherichia coli* in Conjunction with Cord Blood Neutrophils and Sera, but Not with Spinal Fluid

ALAN S. CROSS,¹⁷ WILLIAM H. WOOLDRIDGE, AND WENDELL D. ZOLLINGER

Department of Bacterial Diseases, Walter Reed Army Institute of Research and Department of Pediatrics, Walter Reed Army Medical Center, Washington, DC, USA

Summary

In conjunction with 400 ng/ml murine monoclonal antibody of the IgM class, cord neutrophils were able to kill K1-positive *Escherichia coli* in an *in vitro* opsonophagocytic assay. Neither adult nor cord neutrophils mediated bacterial killing in the absence of this monoclonal antibody. This bactericidal capacity was observed with eight of 11 (73%) but not all cord neutrophil samples, and under conditions of bacteria to neutrophil ratios as high as 15:1. Increasing the monoclonal antibody concentration up to 12 µg/ml paradoxically resulted in significantly lower amounts of bacterial killing. All 11 cord serum samples had sufficient complement activity to permit this monoclonal antibody to function; however, neonatal cerebrospinal fluid was not an effective complement source. We conclude that this monoclonal antibody may be a useful adjunct to conventional therapy of invasive disease caused by K1-positive *E. coli*; but it would have to work at the bacteremic phase of infection.

Abbreviation

PMN, polymorphonuclear leukocytes

Escherichia coli encapsulated with the K1 polysaccharide are the most common cause of neonatal meningitis due to gram-negative bacilli, and also account for approximately 20% of *E. coli* isolates obtained from the blood of adults with bacteremia (9, 11). Because of the difficulty of both normal host immune defenses and antibiotics to adequately clear the infection, augmentation of the immune response as a supplement to current therapeutic regimens has been an attractive approach. Unfortunately, neither purified K1 polysaccharide, which is a polymer of sialic acid, nor whole K1-positive *E. coli* elicits a significant antibody response in man (4, 6). Consequently, neither active elicitation nor passive administration of antibody to the K1 capsule appears to be a feasible therapeutic strategy.

We have previously reported that an immunoglobulin M class monoclonal antibody is highly protective for mice lethally infected with K1-positive *E. coli*. Further, in combination with adult human neutrophils and adult human fresh sera this monoclonal antibody can kill K1-positive *E. coli* with as little as 5 ng antibody protein/ml under conditions where normal human sera is unable to effect any bacterial kill (4). Since the morbidity and mortality from infection with K1-positive *E. coli* occur primarily in the neonatal age group, we sought to determine whether this monoclonal antibody could also function with cord neutrophils. Finally, since this antibody requires complement for its function (4), we measured whether cord blood serum and neonatal spinal fluid had sufficient functionally active complement. Our data demonstrate that cord blood neutrophils in 73% (8 of 11) cord

samples tested can kill K1-positive *E. coli* with this monoclonal antibody. Finally cord blood, but not neonatal spinal fluid, has sufficient active complement for the monoclonal antibody to function.

MATERIALS AND METHODS

Monoclonal antibody preparation. We took advantage of the previously known immunochemical cross-reactivity between the K1 capsular polysaccharide of *E. coli* and the capsular polysaccharide of group B meningococcus to prepare a monoclonal antibody that reacts with both capsular antigens, as previously described (4, 6). Briefly, mice were immunized with live group B, type 15 meningococci. Fusion of a P3-derived nonproducer mouse myeloma cell line (X63-Ag8.653) and spleen cells from the immunized BALB/cJ mice was done according to the procedure described by Kennett (7). Cells from clones that secreted antibody specific for the capsular polysaccharide were injected into Pristane-primed mice. This resulted in ascites fluid that contained 5 to 15 mg of antibody/ml that was of the immunoglobulin M class and specific for the two cross-reactive capsules.

Opsonophagocytic test. The assay of Hirsch and Strauss adapted to a microtiter-system by Sadoff *et al.* (13) has been previously described. Whole blood was obtained from both normal human adult volunteers and from cord blood obtained from elective cesarean section deliveries. Aliquots of blood were allowed to clot and the resulting serum preserved at 0°C to retain complement activity until tested that same day. Another aliquot of blood was sedimented in dextran, treated with Ficoll-Hypaque and subjected to hypotonic lysis. The neutrophils, of which 98% excluded trypan blue, were adjusted to a concentration of 20×10^6 /ml. Cord and adult blood were processed simultaneously.

Neutrophils (1.4×10^6), complement (10% final concentration), monoclonal antibody (10 µl), and bacteria (1×10^6) were added to a total volume of 100 µl. Samples were obtained for colony counts at 0 and 120 min. A clinical isolate of K1-positive *E. coli*, E781 (01:K1:H⁻) was the test of organism. A serum-sensitive *E. coli* K12 strain, C600, was used to monitor complement activity. A serum-resistant *E. coli*, ONKN (kindly provided by Dr. Paul Quie, Minneapolis, MN), served as a phagocytosis-positive control.

Spinal fluid was obtained from neonates and adult patients being evaluated for fever. Samples were promptly frozen at -20°C and kept until use in the assay.

RESULTS

Adult neutrophils with fresh or adult normal human serum (20%), or cord neutrophils with fresh cord human serum were unable to kill K1-positive *E. coli* (Table 1). When 400 ng/ml

monoclonal antibody and adult neutrophils were added to either adult or cord sera, over 90% of the original bacterial inoculum was killed (Table 1). This indicates that both adult and cord sera had sufficient complement activity to permit the monoclonal antibody and neutrophils to kill the bacteria.

The same monoclonal antibody was able to kill K1-positive *E. coli* with cord neutrophils, although not as consistently as with adult neutrophils. When combined with cord sera and monoclonal antibody, 73% of cord neutrophil preparations killed in excess of 70% of the initial bacterial inoculum. With both sets of neutrophils, cord sera appeared to be as effective as adult sera as a complement source when optimal amounts of monoclonal antibody were used. At suboptimal concentrations of monoclonal antibody, cord sera may be less effective than adult sera.

In subsequent experiments, we subjected the neutrophils to various conditions of stress (by increasing the amount of bacteria while maintaining the same number of neutrophils, or by maintaining the same number of bacteria while decreasing the number of neutrophils). In three subsequent experiments with different neutrophil preparations, we found that cord neutrophils were consistently able to kill bacteria up to ratios of 14 bacteria to one neutrophil when monoclonal antibody and cord serum were used. Higher amounts of monoclonal antibody actually resulted in less effective kill of the bacteria (Table 2).

Cord neutrophils were able to kill the bacteria in conjunction with monoclonal antibody and fresh cord sera at neutrophil

concentrations as low as 1.25×10^6 /ml. Although at this concentration of neutrophils, the bacteria were killed less effectively, higher concentrations of antibody again resulted in less effective kill of the bacteria (Table 3).

In order to see if neonatal spinal fluid had sufficient functionally active complement such that the monoclonal antibody might be able to kill *E. coli* when present at this site of infection, we substituted neonatal spinal fluid in place of sera in our phagocytic assay. In no instance was the spinal fluid able to support the kill of the bacteria by neutrophil and monoclonal antibody (Table 4). Further, we were unable to detect functionally active complement in two other assays. Finally, to exclude the possibility of improper handling of spinal fluid as a cause for lack of complement activity, we tested for the presence of antigenic C3 and C4 on radial immunodiffusion plates. This method is able to detect these components in either the active or inactive form. In each instance, again, there was little activity of either C3 or C4 in neonatal spinal fluid, but ample amounts were found in neonatal sera. This lack of functional and antigenic complement was also found in five samples of spinal fluid from adult patients.

DISCUSSION

Our results show that in conjunction with monoclonal antibody and complement, cord neutrophils can kill K1-encapsu-

Table 1. Bacterial killing of K1-positive *Escherichia coli* in vitro by neutrophils (P.M.N.), monoclonal antibody, and fresh serum

PMN	Serum	Monoclonal antibody*		
		400 ng/ml	120 ng/ml	0
Adult	Adult	95 ± 2 (84-100)	94 ± 3 (84-100)	0
	Cord	95 ± 2 (74-100)	79 ± 10 (48-100)	ND†
Cord	Adult	61 ± 13 (0-100)	55 ± 19 (0-98)	ND
	Cord	70 ± 12 (0-100)	40 ± 23 (0-99)	0

* Percentage of original bacterial inoculum killed ± SEM. Eleven separate pairs of adult and cord blood samples (i.e., PMN and sera) were analyzed in duplicate at 400 ng/ml and four separate pairs were analyzed at 120 ng/ml.

† ND, not done.

Table 2. In vitro opsonophagocytic killing of K1-positive *Escherichia coli* with increasing concentrations of bacteria

Bacteria ($\times 10^6$ /ml)	Cord PMN ($\times 10^6$ /ml)	Amount of monoclonal antibody added (ng/ml) to cord sera	% kill*
27	10	400	99.5 ± 0.5 (99-100)
		1,200	76 ± 20 (0-100)
		2,400	61 ± 20 (0-94)
56	10	400	96 ± 0.7 (95-96)
140	10	400	77 ± 3 (75-79)
		1,200	90 ± 4 (87-93)
		2,400	37 ± 4 (34-40)

* Numbers represent mean ± SEM of three separate experiments done in duplicate with bacteria at 27×10^6 /ml. Percent kill with bacteria at 56 and 140×10^6 /ml represent results from single experiment performed in duplicate.

Table 3. In vitro opsonophagocytic killing of K1-positive *Escherichia coli* by decreasing concentrations of cord PMN

Bacteria ($\times 10^6$ /ml)	Cord PMN ($\times 10^6$ /ml)	Amount of monoclonal antibody added (ng/ml) to cord sera	% kill*
10	10	400	80 ± 16 (73-100)
		2,400	60 ± 20 (25-98)
		12,000	17 ± 10 (0-46)
10	1.25	400	69 ± 22 (0-96)
		2,400	30 ± 23 (0-99)
		12,000	0

* Numbers represent mean ± SEM from four separate experiments done in duplicate.

Table 4. Ability of either cord serum or spinal fluid to serve as source of functionally active complement*

	Cord sera	Spinal fluid
Lysis of sensitized sheep red blood cells	+	-
Ability to mediate kill of serum-sensitive <i>Escherichia coli</i> (with heated NHS)	+	-
Ability to mediate kill of K1+ <i>E. coli</i> with adult PMN + monoclonal antibody	+	-
C3 level (mg/100 ml)	104 ± 30 (69-121)	<1
C4 level (mg/100 ml)	20 ± 7 (14-27)	<1

* Lysis of sensitized sheep red blood cells performed by radial diffusion with Quantiplate Complement Test Kit (Kallestad, Austin, TX). C3 and C4 levels are also performed by radial immunodiffusion with C3 and C4 Complement Test Kit (Kallestad). + = 5 of 5 samples reacted in indicated test; - = 4 of 4 samples from neonates and 5 of 5 samples from adults who were being evaluated for fever did not react in indicated test. Both neonatal and adult spinal fluid had no detectable levels of C3 or C4.

lated *E. coli*. Further, cord blood has sufficient functional complement activity for the monoclonal antibody to promote the uptake and kill of these bacteria. This murine monoclonal antibody is potent (just 5 ng/ml is sufficient) and protects mice *in vivo* from lethal challenge with K1-positive *E. coli* (4).

Sepsis neonatorum due to *E. coli* has a 20–40% mortality, even with optimal antibiotic therapy. It has been suggested that passive therapy with immunoglobulin modified for intravenous use (IISG) might be useful in the treatment of some infectious diseases of the neonate (14). Preparations of IISG, however, have low levels of antibody directed at the K1 capsule of *E. coli* (4). This reflects the low levels of such antibody in the general population. Thus, therapeutic strategies aimed at immunologic manipulation are hampered by the lack of immunogenicity of the K1 capsule. Consequently, the availability of a high-titered, functionally active monoclonal antibody may be useful in the treatment of this disease.

Defects in cord and neonatal neutrophil function have been well described. A cellular and humoral defect in the chemotactic response (2), altered ability to produce leukocyte pyrogen (5), abnormal adherence, and decreased viability (8, 15) have all been reported. In contrast, the uptake and killing of bacteria is normal in neutrophils of well neonates. With illness, however, these functions may also become compromised (16). Granulocyte transfusions can improve the outcome in neonatal sepsis, indicating that a relative impairment of neonatal neutrophil function does occur during illness (10).

Nearly 75% of the cord neutrophil samples we examined were able to kill the K1-positive *E. coli* in cooperation with the monoclonal antibody. The inability of some cord neutrophil samples to kill the bacteria may have been due to the adverse effects on neutrophils of medications administered to the mother before or during delivery, an ill neonate, or a suboptimal laboratory preparation of cord neutrophils. Although it was not the purpose of this study to define the conditions under which cord neutrophils can optimally function, this study does establish that cord neutrophils can cooperate with this monoclonal antibody.

Our data further demonstrate that under conditions of neutrophil stress *in vitro* (i.e., high bacteria to neutrophil ratios or low absolute numbers of neutrophils) cord neutrophils still function effectively. The additions of greater amounts of antibody does not result in improved bacterial kill, however, but paradoxically in less kill. This observation is similar to our earlier observations that murine monoclonal antibody in concentrations in excess of 40 µg/ml was unable to kill K1-positive *E. coli*. The mechanism of this prozone phenomenon is unclear.

While the levels of complement in neonatal blood are 60–80% of those of adults, all cord sera that we examined, regardless of neutrophil defects, had sufficient complement to permit effective bactericidal activity by monoclonal antibody and adult neutrophils. In contrast, all samples of spinal fluid lacked complement activity. This lack was not due to improper handling of the spinal fluid since there was no evidence of even inactivated C3 on radial immunodiffusion assay. This suggests that if this monoclonal antibody is to be used *in vivo* it must be at the bacteremic phase of infection. In addition, immunoglobulin M class of antibody

such as this murine monoclonal antibody diffuses poorly into the spinal fluid (1).

Monoclonal antibodies of murine origin have been given to human adults (3, 12). Several problems must be considered in giving this monoclonal antibody to neonates. The short and long term safety for neonates of monoclonal antibody therapy has not been established. Finally, sialic acid is a normal component of normal human tissue. It is not clear whether antibody directed against the sialic acid of a bacterial capsule will also adversely bind to host tissues. Further evaluation of this antibody is therefore needed.

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17. Request for reprints should be addressed to: Alan S. Cross, M.D. Department of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, DC 20307.
18. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense.
19. We appreciate the expert technical assistance of Joseph O'Brien and Lynnette Young. Jean Guidas provided invaluable help in the preparation of this manuscript.
20. Received for publication November 29, 1983.