Pediat. Res. *13:* 1093–1096 (1979)

Bacterial infection host defense
complement neonates

Defective Activation of the Third Component of Complement in the Sera of Newborn Infants

JERRY A. WINKELSTEIN, LAWRENCE E. KURLANDSKY, AND ANDREA J. SWIFT

The Howard Hughes Medical Institute Laboratory, Departments of Pediatrics and Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Summary

The activation of the terminal complement components, C3–9, plays an important role in the host's defense against infection. In the present study, the ability of bacteria to activate the third component of complement (C3) in newborn serum was examined.

A variety of bacteria were incubated in test sera at 37°C for 30 min and the percent of available C3 that was activated was measured. Using one strain of *Escherchia coli* (#3), 32% (mean) of the available C3 was activated in sera from 18 newborns, as compared to 85% in sera from their mothers and 79% in sera from 13 normal adults ($P < 0.005$). In contrast, using another strain of *E. coli* (N70), the percent of C3 activated in newborn sera (83%) was the same as in sera from their mothers (81%) or in sera from normal adults (73%). The defective activation of C3 in newborn sera by *E. coli* was not related to the presence of the K1 antigen. Newborn sera were also challenged with other bacterial species and the activation of C3 was deficient when tested with klebsiellae, but not with staphylococci or streptococci. The defect in newborn sera appeared to be due to a deficiency of a serum factor rather than to the presence of an inhibitor.

Speculation

The defective activation of C3–9 in newborn sera by some bacteria may be, in part, responsible for the neonate's increased susceptibility to infection.

The terminal complement components (C3–9) play an important role in the host's defense against bacterial infections (5). However, in order to subserve their protective functions, C3–9 must first be activated to produce biologically active products. Activation of C3–9 by either the classical or the alternative pathway leads to the generation of chemotactic, opsonic, anaphylatoxic, and bactericidal activities.

Newborn infants are unusually susceptible to frequent and severe bacterial infections. A number of defects in host defense have been described in these infants, each of which could contribute to their increased susceptibility to infection (9). Among these, deficiencies in both serum chemotactic and opsonic activity have been identified in newborns (2, 4, 7, 8, 12). Because these two serum activities are, at least in part, dependent on the activation of C3–9, the previously mentioned observations suggest that the activation of the terminal complement components might be defective in newborn sera.

Accordingly, in the following experiments, the ability of a variety of pathogenic bacteria to activate C3 in newborn sera was studied.

MATERIALS AND METHODS

SERA

Sera were obtained from the cord blood of 18 normal full term neonates at the time of their birth and from their mothers within 12 hr of delivery. Sera were also obtained from 13 normal adult males and females. Informed consent was obtained. Each sample was divided into small aliquots, frozen, and stored at −70°C.

BUFFERS

Veronal buffered saline, pH 7.4, ionic strength 0.147, with 0.15 mM Ca^{++}, 1 mM Mg^{++}, and 0.1% gelatin (GVB^{++}) was prepared according to the method of Kabat and Mayer (6). Veronal buffered saline, pH 7.4, ionic strength 0.074, with 0.15 mM Ca^{++}, 1 mM Mg^{++}, 0.1% gelatin, and 2.5 % dextrose ($DGVB^{++}$) was prepared according to the method of Shin and Mayer (11).

BACTERIA

The strains of *Staphylococcus aureus*, *Klebsiella pneumoniae* and group B β hemolytic streptococci were isolated from the blood of infected neonates at the Johns Hopkins Hospital. The strains of *E. coli* were isolated from the blood or cerebrospinal fluid of infected neonates and were kindly supplied by Dr. John Robbins. the Bureau of Biologics, Food and Drug Administration. Bethesda, MD. They were tested for the presence of the K1 antigen by their reaction with cross reacting antiserum to type B meningococcus on solid media (10). The bacteria were grown in Trypticase Soy broth at 37°C for 18 hr, washed three times in GVB^{++}, and suspended to the desired concentration in GVB^{++}.

C3 ACTIVATION

The ability of bacteria to activate C3 in whole serum was tested as previously described (13). The appropriate organism was suspended to the desired concentration in test serum that had been diluted 1/10 in GVB^{++}, and the mixture incubated at 37°C. After 30 min, a sample was taken, diluted immediately in ice-cold $DGVB^{++}$ and the titer of functionally active C3 determined using a hemolytic assay (11). The amount of C3 consumed in each test sample was expressed as a percent of that amount of C3 remaining in a control sample of the test serum incubated with buffer alone.

RESULTS

ACTIVATION OF C3 IN NEWBORN SERA BY VARIOUS STRAINS OF *E. COLI*

E. coli, strain #3 (01:K1:H-), were incubated at a concentration of 6×10^8/ml in the sera from newborn infants, their mothers, or normal adults and the consumption of C3 determined. As can be seen in Figure 1, when newborn sera were challenged with this strain of *E. coli*, there was significantly less consumption of the available C3 than when sera from either their mothers or normal adults were challenged ($P < 0.005$ using the Student's t test).

In contrast, when the same sera were challenged with a different strain of *E. coli*, strain N70 (023:K22:H15), approximately the same percentage of the available C3 was consumed in the newborn sera as in the sera from their mothers or normal adults (Fig. 2).

The previous two series of experiments suggested that the defective activation of C3 in newborn sera by one strain of *E. coli*

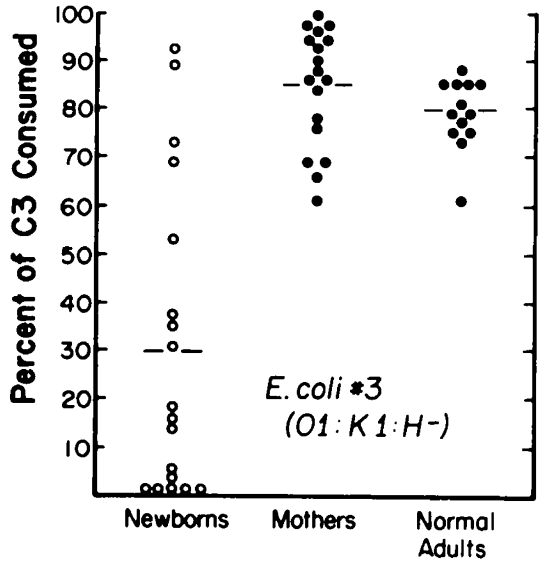

Fig. 1. The activation of C3 in a variety of sera by *E. coli*, #3. The mean for each group is represented by the *horizontal line*.

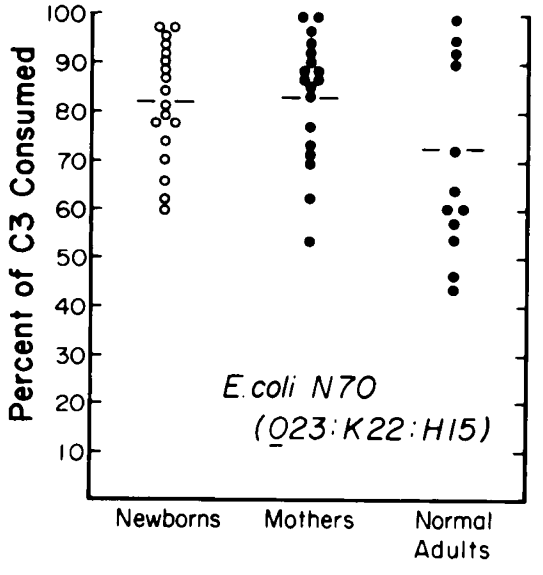

Fig. 2. The activation of C3 in a variety of sera by *E. coli*, N70. The mean for each group is represented by the *horizontal line*.

(K1 positive), but not the other (K1 negative), might be related to the presence of the K1 antigen. In order to examine that possibility, the serum from a single neonate and its mother were challenged with different strains of K1 positive and K1 negative *E. coli* over a wide range of bacterial concentrations. As can be seen in Figure 3, two of the K1 positive strains of *E. coli* gave activation of C3 in neonatal serum comparable to that in maternal serum, whereas two other K1 positive strains gave significantly less activation of C3 in the neonatal serum than in the maternal serum. As can be seen in Figure 4, two of the K1 negative strains of *E. coli* gave activation of C3 in the newborn serum comparable to that in the maternal serum, whereas two other K1 negative strains gave significantly less consumption of C3 in neonatal serum than in maternal serum. Comparable results were obtained when other neonatal-maternal serum pairs were tested in a similar manner. Thus, the defective activation of C3 in neonatal serum by some strains of *E. coli*, but not others, does not appear to be solely related to the presence of the K1 antigen.

ACTIVATION OF C3 IN NEWBORN SERA BY OTHER BACTERIA

The following experiments were performed in order to determine if the activation of C3 in neonatal sera was defective when tested with other species of bacteria. When newborn sera were challenged with 6×10^8 klebsiellae/ml, there was significantly less consumption of C3 than when maternal sera were challenged ($P < 0.01$) (Fig. 5). In contrast, when the same neonatal sera were challenged with either group B streptococci or staphylococci at a concentration of 6×10^8/ml, the percent of available C3 consumed was the same as that in the maternal sera (Fig. 5).

EFFECT OF NEWBORN SERUM ON THE CONSUMPTION OF C3 IN MATERNAL SERA

The following experiments were performed in order to determine if the defective activation of C3 in neonatal serum was due to the presence of an inhibitor or due to a deficiency of a serum factor or factors. As can be seen in Table 1, when three different neonatal sera were challenged with *E. coli* #3 there was little, if any, consumption of the available C3, whereas the challenge of the maternal sera resulted in marked consumption of the available C3 in each case. When mixtures of neonatal and maternal sera were challenged, there was also marked consumption of the available C3. Thus, the defective activation of C3 in neonatal sera seemed to be due to a deficiency of a serum factor or factors, rather than to the presence of an inhibitor.

DISCUSSION

Activation of the terminal complement components, C3–9, results in the production of serum opsonic, anaphylatoxic, chemotactic, and bactericidal activities, all of which may serve to protect the host in its defense against infection (5). At least two mechanisms exist by which bacteria may activate C3–9, the classical and the alternative pathway. Activation of the classical pathway by bacteria requires the participation of antibody. In contrast, although antibody is able to participate in the activation of the alternative pathway by bacteria (14) little, if any, antibody is actually required. Which of these two pathways an individual strain or species of bacteria uses to activate C3–9 probably depends on a number of different factors such as the presence or absence of antibody, the relative concentrations of classical and alternative

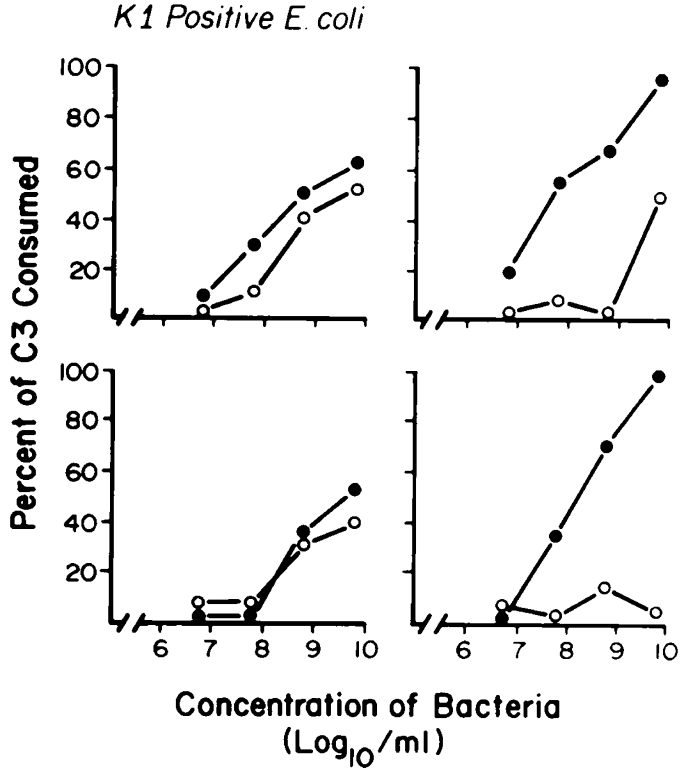

Fig. 3. The activation of C3 in the serum from a newborn infant (○) and its mother (●) by four different strains of K1 positive *E. coli*.

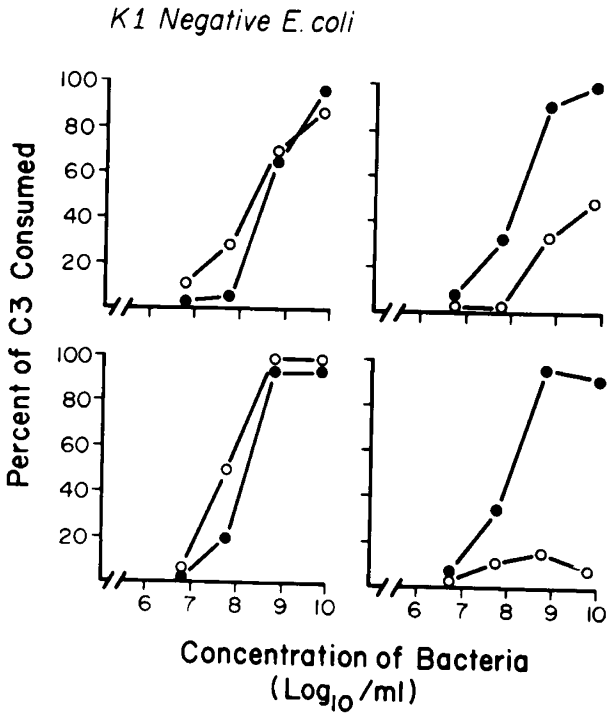

Fig. 4. The activation of C3 in serum from a newborn infant (○) and its mother (●) by four different strains of K1 negative *E. coli*.

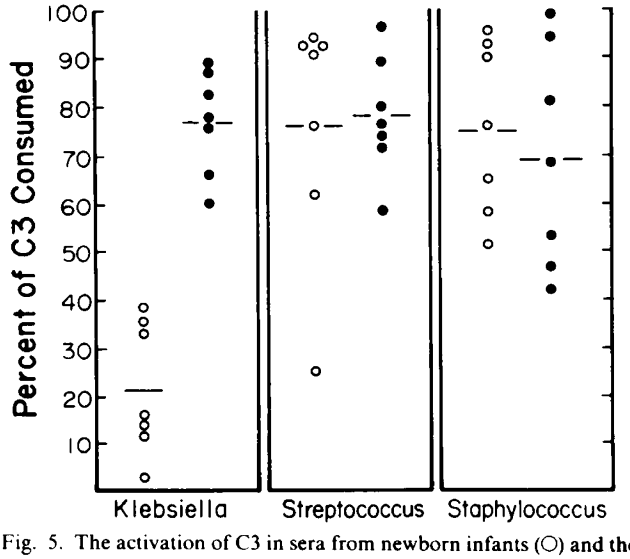

Fig. 5. The activation of C3 in sera from newborn infants (○) and their mothers (●) by a variety of bacterial species. The mean for each group is represented by the *horizontal line*.

Table 1. *The effect of newborn serum on consumption of C3 in maternal serum by E. Coli #3*

Sera	Percent of C3 Consumed		
	Exp #1	Exp #2	Exp #3
10% newborn	0	0	17
10% maternal	100	87	82
10 % newborn + 10% maternal	87	100	84

pathway components and the intrinsic ability of the bacteria to activate the alternative pathway.

A number of studies have been performed on the complement system of newborns. Levels of the individual components of the classical complement system, C1-9, have been found to be significantly depressed in the cord blood of neonates (1). In addition, one of the components of the alternative pathway, factor B, has been shown to be decreased in neonates (3, 12).

The present experiments were designed to study the ability of bacteria to activate C3 in newborn sera. The bacteria selected for use were chosen because they are common pathogens in the newborn period. The ability of these bacteria to activate C3 was studied because C3 itself plays an important role in the host's defense against infection (15), and also serves as part of the enzyme that activates C5-9 (5). No attempt was made in the present experiments to study the activation of C3 exclusively by either the classical or alternative pathway. Rather, the total amount of C3 activated by both pathways was measured in each serum sample because it more accurately reflects the situation as it occurs *in vivo*.

In the present study, the activation of C3 in newborn sera was found to be deficient when tested with two different gram-negative species, *E. coli* and klebsiella. In contrast, when these same sera were tested with two different gram-positive organisms, staphylococcus and group B streptococcus, the activation of C3 was normal. In addition, not all strains of *E. coli* were found to cause defective activation of C3 in newborn sera. The results, however, for a given species and strain of bacteria were consistent from infant to infant. The reason(s) why some bacteria cause defective activation of C3 in newborn sera and others do not is unknown. In the case of *E. coli*, the results of the present experiments suggest that the defective activation of C3 is not solely due to the presence of the K1 antigen.

The defect or defects in newborn sera responsible for the deficient activation of C3 are unknown. The results of the present study would suggest, however, that the defective activation of C3 is due to a deficiency of a serum factor or factors, rather than to the presence of an inhibitor. The defective activation of C3 by some bacteria, but not by others, may be related to a number of variables such as the relative integrity of the classical and alternative pathways in newborn sera, the absence of IgM in newborn sera, or the relative abilities of the different bacteria to activate each pathway. The fact that some bacterial species and strains are able to activate C3 normally in newborn sera implies that at least one activation mechanism is intact in newborn sera.

The results of the present study demonstrate that the activation of C3 in neonatal serum is defective when tested with some, but not all, bacteria. Because the activation of C3-9 plays an important role in the host's defense against infection, it is possible that the defective activation of C3 in newborn serum contributes to the neonate's remarkable susceptibility to bacterial infection.

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16. This research was supported, in part, by United States Public Health Service Grant AI-11637. Jerry A. Winkelstein is an Investigator of the Howard Hughes Medical Institute.
17. Requests for reprints should be addressed to: Jerry A. Winkelstein, M. D., Department of Pediatrics, The Johns Hopkins Hospital, 600 N. Wolfe Street, Baltimore, MD 21205, USA.
18. Received for publication August 4, 1978.
19. Accepted for publication October 24, 1978.

0031-3998/79/1310-1093$02.00/0

Printed in U. S. A.