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Effect of Anti-B Antiserum on the Phagocytosis of *Escherichia coli*

JEROME H. CHECK,[1] ELIZABETH A. O'NEILL, KAREN E. O'NEILL, AND KATHRYN E. FUSCALDO

Department of Radiation Therapy and Nuclear Medicine, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania 19102

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An increase in phagocytosis of *Escherichia coli* O86, an organism containing blood group B substance, was seen in the presence of anti-B antibodies.

The presence of blood group antigens in several pathogenic microorganisms has been demonstrated (2, 3). *Escherichia coli* O86 has been shown to have one of the highest blood group B activities of any known microorganism (1). Muschel showed an increase in bactericidal reaction against *E. coli* O86, with both human and rabbit sera having anti-B activity, and showed that absorption of the rabbit sera with human group B erythrocytes considerably reduced the bactericidal titer (1). The present study shows that anti-B antibodies will significantly opsonize *E. coli* O86 and that absorption of the anti-B antibodies causes a significant decrease in phagocytosis.

High-titered (saline antibody titer = 256), pooled human anti-A and anti-B antisera were used (obtained from Hyland Laboratories, Calif.). The antisera did not contain indicator dyes, and the preservative (sodium azide) was removed by dialysis. We attempted to remove "natural" *E. coli* antibodies by absorbing the antisera with a heat-killed 1×10^8 suspension of *E. coli* O26, an organism which does not contain the blood group substance (4).

E. coli O86 was grown in synthetic medium devoid of any blood group substance (Difco minimal agar Davis). The bacteria were incubated at 37 C for 24 hr prior to use. The plasma from type "O" blood was removed, and the packed red blood cells were washed three times in saline. A 0.2-ml sample of the plasma-free cellular suspension in saline was added to each of eight tubes, and a 0.1-ml culture of *E. coli* O86 (5×10^8 bacteria per ml) was added to each tube. The following antisera were then added to the tubes: 0.1 ml of anti-A, 0.1 ml of anti-B, 0.1 ml of anti-A (absorbed with type "A" erythrocytes), 0.1 ml of anti-A (absorbed with type "B" erythrocytes), 0.1 ml of anti-B (absorbed with type "A" erythrocytes), 0.1 ml of anti-B (absorbed with type "B" erythrocytes), 0.1 ml of anti-A (absorbed with *E. coli* O86),

TABLE 1. *Effect of blood group antisera on the phagocytosis of Escherichia coli in vitro*

Group	Titer (anti-A or anti-B)	Avg no. of bacteria per neutrophile			
		Expt 1	Expt 2	Expt 3	Mean
Anti-A	1:256	1.82	0.98	0.78	1.19
Anti-B	1:256	13.03	9.71	10.00	10.91
Anti-A absorbed with A cells	1:16	4.53	1.68	1.05	2.42
Anti-A absorbed with B cells	1:256	1.87	1.27	1.03	1.39
Anti-B absorbed with A cells	1:256	10.86	7.94	9.67	9.49
Anti-B absorbed with B cells	1:16	3.50	3.10	2.97	3.19
Anti-A absorbed with *Escherichia coli* O86	1:256	1.23	1.84	1.32	1.46
Anti-B absorbed with *E. coli* O86	1:64	5.60	4.80	4.50	4.96

[1] Present address: Lankenau Hospital, Philadelphia. Pa,

and 0.1 ml of anti-B (absorbed with *E. coli* O86).

The mixtures were incubated at 37 C for 35 min with agitation. Blood smears were then made from each sample and stained with Wright's stain. The slides were examined with a microscope for the presence of bacteria in the neutrophiles. To establish a relative count, the first 100 neutrophiles on each slide were counted, and the results were recorded (Table 1).

In all three experiments in which anti-B serum was used, there was a significant increase in the phagocytosis of bacteria, although not to the same extent in each case. The unabsorbed anti-B serum was most effective in stimulating phagocytosis (10.91 bacteria per neutrophile). When the anti-B serum was absorbed with type "B" cells, the increase in phagocytosis was considerably lower (3.19 bacteria per neutrophile). Absorption of anti-B serum with *E. coli* O86 produced an effect comparable to that obtained with type "B" red blood cells, although not as marked (4.96 bacteria per neutrophile). The *E. coli* O86 was not quite as effective in absorbing anti-B sera as the "B" type red blood cells. The discrepancy may be more apparent than real and may be due to our inability to decrease the anti-B titer by absorption with *E. coli* O86 to the same level as with type "B" cells. Present experiments are aimed at determining whether this significant opsonizing effect of anti-B antibodies has any protective effect in vivo.

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