ASSOCIATED LEUKOCYTE RESPONSES IN THE LETHAL ASPECTS OF *E. COLI*.

APR 77  L B WINSHAW, B K BELLER, L T ARCHER

UNCLASSIFIED

END

DATE FILMED 6-77
ASSOCIATED LEUKOCYTE RESPONSES IN THE LETHAL ASPECTS OF E. COLI SHOCK

L. B. Hinshaw, B. K. Beller, L. T. Archer, and G. L. White

Prepared for Publication in
Proceedings of the Society for Experimental Biology and Medicine

University of Oklahoma Health Sciences Center
Departments of Physiology and Biophysics and Research Surgery
Oklahoma City, Oklahoma

15 April 1977

Reproduction in whole or in part is permitted for any purpose of the United States Government
Distribution of this report is unlimited
ASSOCIATED LEUKOCYTE RESPONSES IN THE LETHAL ASPECTS OF E. COLI SHOCK

B. Hinshaw, R. K. Beller, T. Archer, G. L. White

Prepared for Publication
in
Proceedings of the Society for Experimental Biology and Medicine

University of Oklahoma Health Sciences Center
Departments of Physiology and Biophysics and Research Surgery
Oklahoma City, Oklahoma

Reproduction in whole or in part is permitted for any purpose of the United States Government
Distribution of this report is unlimited
Dogs administered lethal injections of *E. coli* endotoxin or *E. coli* organisms develop systemic hypotension, hypoglycemia and hepatosplanchnic dysfunction (1-4). Progressively decreasing blood glucose levels after endotoxin or *E. coli* administration are due in large part to depressed hepatic function, particularly gluconeogenesis (4-7). Accelerated glucose uptake has been reported following *in vitro* incubation of either endotoxin or live *E. coli* organisms in blood, and white blood cell (WBC) phagocytic activity has been implicated as the primary responsible factor (2). Increased phagocytic activity of the blood after endotoxin (8) has been traced to the buffy coat (9) and the leukocyte (10). Recent reports have shown circulating neutrophils to be of major importance in the clearance of bacterial organisms (11) or endotoxin (12) from the blood, while others have described beneficial effects of transfused WBC's in patients and animals in septic shock (13,14).

The purpose of the present study was to explore the responses of canine blood to the separate effects of *E. coli* organisms and *E. coli* endotoxin, particularly emphasizing the role of the WBC in the uptake of glucose *in vitro* and its possible relationship to survival *in vivo*.

**Materials and methods.** *In vivo* experiments were carried out on twelve awake adult mongrel dogs during a 4-day period. On the fourth day, venous blood was drawn from each animal and additionally studied in the *in vitro* state. Animals, selected for robust health and absence of heart worms, were treated for intestinal parasites and conditioned in the animal facility for 3-6 weeks prior to use. Dogs with initial WBC counts between 7,000 and 20,000/mm$^3$ and hematocrits exceeding 37% were utilized in the experiments.
In vivo studies. Unanesthetized, gently restrained animals were divided into paired control and experimental groups which were studied simultaneously. The experimental group received sublethal doses of endotoxin (Difco, Detroit); 1/1,000 LD100 on days 1 and 2 (0.003 mg/kg body weight), 1xLD100 on day 3 (3 mg/kg), followed by 2xLD100 live E. coli on day 4 (2.5x10^10 organisms/kg). The control group received equal volumes of saline on days 1, 2 and 3, and on day 4 received the identical dose of E. coli organisms as in the experimental group. The LD100 of E. coli endotoxin and E. coli organisms was previously established in this laboratory. Animals living 6 days following injection of E. coli were considered permanent survivors.

In vitro studies. An in vitro system served as a test device to assay responses of the blood to E. coli endotoxin and E. coli organisms in the absence of the organs of gluconeogenesis and with the prevention of the cell migration which occurs in vivo. Accelerated uptake of glucose by the blood, ascribed to increased metabolic activity of white blood cells, was described in an earlier study (2). Blood for in vitro studies was drawn intravenously from the twelve awake dogs on the fourth day prior to their receiving E. coli injections and incubated as previously reported (2). Three tubes of blood obtained from each control (saline-pretreated) and experimental (sublethal endotoxin-pretreated) animal were studied in vitro following separate additions of E. coli or endotoxin, at LD100 doses, or saline.

WBC counts were measured with an automatic particle counter (Coulter ZF; Hialeah, Florida) and the differential WBC by microscopic examination of blood stained with Wrights stain. Blood
glucose concentrations were determined with a Beckman Glucose Analyzer (Beckman Instruments; Fullerton, Calif.) possessing an accuracy of ±3 mg%. Venous blood samples for in vivo studies were placed in vacutainers containing ethylenediamine-tetraacetic acid (EDTA; Beckton-Dickinson). Blood samples for in vitro studies in 10 ml volumes were anticoagulated with heparin (0.1 ml; 20,000 units/ml) and incubated in a water bath at 37°-38°C for 3-6 hours. Results from all experiments were analyzed using the t test for paired or unpaired data.

Results. Figure 1 presents in vivo WBC data obtained from animals receiving single sublethal injections of endotoxin on days 1, 2 and 3, and superlethal administrations of E. coli organisms on day 4. Daily values were obtained prior to injections of endotoxin in the experimental group or saline in the control, and values on days 2, 3 and 4 are seen to reflect the effects of the previous injections. Significant leukocytosis (p<0.05) is observed in the experimental group on days 2, 3 and 4, which is accounted for primarily by elevations in blood concentrations of mature and immature neutrophils, while insignificant changes occur in the lymphocyte and monocyte populations. Following injections of 2xLD100 E. coli organisms on the fourth day, leukopenia and neutropenia were observed in experimental and control groups for 2 hours (p<0.01), cell counts showing recovery to near pre-injection values within 6 hours. Mean hourly WBC concentrations in the experimental group during days 1-3 are not shown but by the first hour after sublethal endotoxin administration were lower than the control group (p=0.001) and elevated above it within 6 hours (p<0.05).
All dogs pretreated with sublethal endotoxin survived following superlethal *E. coli* administration while every animal pretreated with saline died within 9 hours after *E. coli* injection, following massive intestinal bloody diarrhea and a protracted moribund condition.

*In vitro* experiments were carried out to determine the effects of *E. coli* endotoxin or *E. coli* organisms on glucose concentrations in blood drawn from animals pretreated with sublethal injections of endotoxin or saline as described above. Samples of blood were withdrawn from animals on day 4 immediately prior to administering superlethal doses of *E. coli* *in vivo*. Figure 2 illustrates the mean results from three paired experiments (total N=36; i.e., 3 sets of 12 experiments each, including control groups). Endotoxin (LD100), *E. coli* organisms (LD100) or saline were added to separate tubes *in vitro* immediately after zero time and observed for 3-5 hours. Mean glucose concentrations are seen to fall significantly below control values in all experiments (p<0.05). *In vitro* glucose concentrations in blood obtained from dogs pretreated with endotoxin *in vivo* were significantly lower than the *in vivo* saline-pretreated group within 60 minutes and were also lower than the group receiving only saline *in vitro* (p=0.05). The endotoxin-pretreated blood groups receiving endotoxin and *E. coli* utilized significantly greater quantities of glucose within 2 hours than the saline groups (p=0.05). The saline-pretreated blood receiving *E. coli* *in vitro* revealed similarly low glucose values by 3 hours. Both the *in vitro* saline control groups and endotoxin group comprised of blood obtained from saline-pretreated animals demonstrated less marked declines in glucose concentrations during the 2-3 hour period. There were no significant
differences between values of the endotoxin-pretreated blood administered endotoxin in vitro and the saline-pretreated blood to which E. coli was added (p>0.05).

It was considered of interest to estimate the rate of glucose uptake per WBC in endotoxin- vs. saline-pretreated blood to which LD100 endotoxin was added in vitro. Previously reported work (2) and parallel studies carried out in this laboratory have implicated the white blood cell as the primary component of blood responsible for increased uptake of glucose following addition of endotoxin in vitro. Washed red blood cells, suspended in a glucose-saline solution, did not demonstrate an increased uptake of glucose following addition of endotoxin in vitro (2). On the basis of these earlier observations, calculations were carried out in the present study to estimate the increased rate of glucose uptake per WBC following addition of LD100 endotoxin in vitro. This excess quantity of glucose was obtained by subtracting the glucose uptake in blood to which saline alone was added from that to which endotoxin was administered. The excess uptake occurring during the first hour was divided by the average WBC count during the same period, in order to estimate the quantity of excess glucose uptake per WBC. Calculations showed the quantity of excess glucose taken up per activated WBC from blood receiving prior sublethal injections of endotoxin was not different from the nonactivated WBC in vitro (11.7x10^-9 vs. 7.4x10^-9 mg glucose/WBC/60 min, respectively) (p>0.05).

Discussion. Progressively developing hypoglycemia in dogs administered endotoxin or E. coli organisms has been documented and found to be associated with systemic hypotension, hepatosplanchnic
pathology and death (1,3). The cause of hypoglycemia has been the subject of much recent research in endotoxin or septic shock. Impaired glucose production as a result of depressed hepatic function has been suggested as a primary factor in the development of hypoglycemia because of adverse effects on gluconeogenesis (4-7). A recent publication from this laboratory suggests that endotoxin also eliminates the gluconeogenic ability of the kidney in the canine species (15).

Recent studies have documented increased uptake of glucose by the blood after endotoxin which partially accounts for the hypoglycemia of shock (2,15). Results from the present study support these earlier observations and further suggest that the accelerated glucose uptake by the blood after endotoxin is primarily due to the increased activity of circulating white blood cells whose rate of glucose utilization varies directly with their total numbers.

Findings from the present investigation suggest a relationship between numbers of white blood cells, particularly neutrophils, and survivability to superlethal doses of E. coli organisms. Daily sublethal intravenous injections of endotoxin administered during a 3-day period resulted in a marked state of leukocytosis. The cause of the elevated numbers of white blood cells was not determined in the present study; however, it is known that endotoxin administration promotes the entry of new leukocytes from the bone marrow into the circulation (16). Animals receiving superlethal injections of E. coli organisms on the fourth day were completely protected against the pathophysiological and lethal effects of the organisms. It is possible that the significantly increased numbers of white blood cells, initially present on the fourth day and
composed primarily of neutrophils, may have efficiently phagocy-
tosed the injected organisms, thereby preserving hepatic function
(7), including gluconeogenesis (6). Additionally, hepatosplanchnic
pooling, extravasation and bloody diarrhea may have been prevented
by augmented white blood cell phagocytotic activity. The degree
of protection seemed remarkable: animals receiving prior sublethal
injections of endotoxin were eating and drinking, appeared normal
in every respect within 12 hours, and all were healthy survivors at
6 days. On the other hand, all animals pretreated only with saline
and challenged on the fourth day with superlethal doses of *E. coli*
uniformly demonstrated the development of massive bloody diarrhea,
vomiting and a subsequent moribund state, dying within 9 hours
post-injection.

The question of possible "activation" of the WBC, in which
each cell becomes more phagocytically active, was not supported by
the results of the present study. Enhanced phagocytic activity
appeared to be due to the increased numbers of white blood cells,
glucose uptake per cell being essentially equal in activated and
nonactivated cells. The WBC types accounting for the total increase
in numbers in the present study were shown to be the mature and
immature neutrophils, cells which have been reported to be particu-
larly active in phagocytosing endotoxin (12) or *E. coli* (11).
Recent studies have documented beneficial effects of transfused
white blood cells in animals and patients in septic shock (13,14).
Results from the present study suggest a relationship between
leukocytosis and survivability in septic shock, lending support to
the view that increased numbers of white blood cells by way of
transfusion may augment the degree of protection.
Bibliography


FIGURE LEGENDS

Figure 1. White blood cell concentrations and differential white blood cell responses to superlethal dose of *E. coli* organisms in dogs following previous sublethal injections of *E. coli* endotoxin (mean±SE; N=6 in each group). The values at the "Control (Days)" time designations are initial measurements recorded prior to injection of endotoxin or saline on days 1, 2 and 3, and *E. coli* organisms on day 4; therefore, the day 4 control value is actually the initial "control" measurement for the values in the "Hours (Day 4)" time designation. The values from 2-8 hours on day 4 are recorded following intravenous administration of *E. coli* organisms; 2xLD$_{100}$ (2.5x10$^{10}$ org/kg). The experimental (endotoxin) group received sublethal doses of *E. coli* endotoxin on days 1 and 2 (1/1,000 LD$_{100}$), on day 3 (LD$_{100}$), and a challenge dose of *E. coli* organisms on day 4 (2xLD$_{100}$). The control (saline) group received equal volumes of saline on days 1, 2 and 3, and on day 4 received 2xLD$_{100}$ *E. coli* organisms. P values represent an unpaired comparison between control and experimental groups.

Figure 2. Effects of *E. coli* organisms (LD$_{100}$) and *E. coli* endotoxin (LD$_{100}$) on blood glucose concentrations in vitro following previous sublethal injections of *E. coli* endotoxin in vivo (N=6 in each group, total N=36). Endotoxin, *E. coli* or saline administered immediately after zero time; LD$_{100}$
endotoxin = $2.5 \times 10^{-2}$ mg/ml blood; LD$_{100}$ *E. coli* organisms = $3 \times 10^8$ organisms/ml blood. Mean glucose concentrations plotted; p values show statistical significances between groups of blood samples obtained from dogs pretreated with endotoxin and saline (see Figure 1 for pretreatment data in *vivo*).
Endotoxin pretreated Dog Blood (N=8)
- Saline
- Endotoxin (2.5 x 10^-2 mg/ml)
- E.coli (3 x 10^8 org/ml)

Saline Pretreated Dog Blood (N=8)
- Saline
- Endotoxin (2.5 x 10^-2 mg/ml)
- E.coli (3 x 10^8 org/ml)

FIGURE 2
The purpose of the present study was to explore the responses of canine blood to the separate effects of E. coli organisms and E. coli endotoxin, particularly emphasizing the role of the WBC in the uptake of glucose in vitro and its possible relationship to survival in vivo.

Results of this study suggest a relationship between leukocytosis and survivability in septic shock, lending support to the view that increased numbers of white blood cells by way of transfusion may augment the degree of protection.
OFFICE OF NAVAL RESEARCH
BIOLOGICAL & MEDICAL SCIENCES DIVISION
MEDICAL AND DENTAL SCIENCES PROGRAM, CODE 444
DISTRIBUTION LIST FOR TECHNICAL, ANNUAL AND FINAL REPORTS

Number of Copies

(12) Administrator, Defense Documentation Center
    Cameron Station
    Alexandria, Virginia 22314

(6) Director, Naval Research Laboratory
    Attention: Technical Information Division
    Code 2627
    Washington, D. C. 20375

(6) Office of Naval Research
    Attention: Code 1021P (ONRL DOC)
    800 N. Quincy Street
    Arlington, Virginia 22217

(3) Office of Naval Research
    Medical and Dental Sciences
    Code 444
    Arlington, Virginia 22217

(1) Commanding Officer
    Naval Medical Research and Development Command
    National Naval Medical Center
    Bethesda, Maryland 20014

(1) Chief, Bureau of Medicine and Surgery
    Department of the Navy
    Washington, D. C. 20375

(2) Technical Reference Library
    Naval Medical Research Institute
    National Naval Medical Center
    Bethesda, Maryland 20014

(1) Office of Naval Research Branch Office
    495 Summer Street
    Boston, Massachusetts 02210

Enclosure (3)
Office of Naval Research Branch Office
536 South Clark Street
Chicago, Illinois 60605

(1)
Office of Naval Research Branch Office
1030 East Green Street
Pasadena, California 91101

(1)
Office of Naval Research
Contract Administrator for Southeastern Area
2110 G Street N.W.
Washington, D. C. 20037

(1)
Commanding Officer
Naval Medical Research Unit No. 2
Box 14
APO San Francisco 96263

(1)
Commanding Officer
Naval Medical Research Unit No. 3
FPO New York 09527

(1)
Officer in Charge
Submarine Medical Research Laboratory
Naval Submarine Base, New London
Groton, Connecticut 06342

(1)
Scientific Library
Naval Medical Field Research Laboratory
Camp Lejeune, North Carolina 28542

(1)
Scientific Library
Naval Aerospace Medical Research Institute
Naval Aerospace Medical Center
Pensacola, Florida 32512

(1)
Commanding Officer
Naval Air Development Center
Attn: Aerospace Medical Research Department
Warminster, Pennsylvania 18974

(1)
Scientific Library
Naval Biomedical Research Laboratory
Naval Supply Center
Oakland, California 94625
Commander, Army Research Office  
P. O. Box 12211  
Research Triangle Park  
North Carolina  27709

Directorate of Life Sciences Div.  
Air Force Office of Scientific  
Research  
Bolling Air Force Base  
Washington, D.C. 20332

Commanding General  
Army Medical Research and Development Command  
Forrestal Building  
Washington, D.C. 20314

Department of the Army  
U.S. Army Science and  
Technology Center - Far East  
APO San Francisco 96328

Assistant Chief for Technology  
Office of Naval Research, Code 200  
800 N. Quincy Street  
Arlington, Virginia 22217