Alterations in Host Defense Associated With Anesthesia and Blood Transfusions

II. Effect on Response to Endotoxin

J. Paul Waymack, MD, ScD; Gabriel Fernandes, PhD; Patricia J. Cappelli; David G. Burleson, PhD; Rey F. Guzman; Arthur D. Mason, Jr, MD; Basil A. Pruitt, Jr, MD

The effect of blood transfusions and anesthesia on host response to endotoxin was evaluated in multiple Lewis rat models. The rats were randomized to receive A'Sogaloff Cancer Institute rat blood, pentobarbital sodium, or lactated Ringer's solution and, at either 2 or 7 days following administration of these agents, were challenged with intravenous endotoxin. Neither blood transfusions nor anesthesia altered mortality when administered 2 days before endotoxin challenge. However, blood transfusions administered 7 days before endotoxin challenge were found to prolong survival, to prevent endotoxin-induced alterations in T-lymphocyte subsets, and to decrease plasma tumor necrosis factor levels. In conclusion, blood transfusions appear to depress immune function in a beneficial manner in endotoxin shock.

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MATERIAL AND METHODS

Rats

A total of 225 adult male Lewis rats weighing approximately 200 g were used for the endotoxin phases of this study. Thirty male A'Sogaloff Cancer Institute (ACI) rats were used as blood donors. The rats were housed in individual stainless steel hanging cages and given food and water ad libitum throughout the study. All rats were observed for 1 week before entry into the study to exclude the possibility of any preexisting diseases.

Anesthesia

Rats that were to receive anesthesia were given pentobarbital sodium (35 mg/kg intraperitoneally [IP]; Vets Lab Limited Inc, Lenexa, Kan). The rats were allowed to recover from anesthesia by breathing room air.

Transfusions

The ACI rat donors were anesthetized with pentobarbital sodium (35 mg/kg IP) and a celiotomy was performed. Blood was withdrawn by vena cava puncture using a 23-gauge needle. The blood was mixed at a 4:1 (vol/vol) ratio with standard CPDA-1 anticoagulant and stored at 4°C for 30 minutes before infusion. The transfusions were given intravenously at a dose of 1 mL per rat, representing 7% of estimated blood volume.
with normal saline to achieve a final concentration of either 10^10 E. coli per milliliter of normal saline. The heat-killed E. coli were administered through a 25-gauge needle into the dorsal penile vein of the rat.

**Endotoxin Mortality**

Ninety additional Lewis rats were divided into six groups of 15. Seven days before endotoxin challenge with 1 x 10^6 heat-killed E. coli, one group received pentobarbital sodium anesthesia (35 mg/kg IP), one group received 1 mL of ACI rat blood intravenously, and one group received 3 mL of lactated Ringer's solution intravenously. Two days before challenge with endotoxin, one group received pentobarbital sodium anesthesia (35 mg/kg IP), one group received 1 mL of ACI rat blood intravenously, and one group received 5 mL of lactated Ringer's solution intravenously. The rats were observed for 7 days following endotoxin challenge to determine mean survival times and absolute survival rates. Previous studies had determined that no further deaths occurred with this model after 7 days. For the calculation of mean survival times, rats surviving 7 days were given a survival time of 7 days.

**Tumor Necrosis Factor (TNF) Production**

Fifteen Lewis rats were divided into three groups of five. Seven days before challenge with endotoxin, one group received pentobarbital sodium anesthesia (35 mg/kg IP), one group received 1 mL of ACI rat blood, and one group received 3 mL of lactated Ringer's solution. Seven days after the administration of these agents, the rats were administered 1 x 10^6 heat-killed E. coli, and 2 hours later were decapitated. Blood was collected in heparinized tubes and plasma was separated by centrifugation. Plasma was assayed for TNF levels using the murine L-929 fibroblast cytotoxicity assay. Briefly, 5 x 10^5 L-929 murine fibrosarcoma cells were incubated in 0.20 mL of complete RPMI solution with a 1:50 dilution of the rat's plasma. The murine fibrosarcomas were cultured for 24 hours at 37°C in 5% carbon dioxide and then were examined to determine the percent cytotoxicity. These values were compared with standards run using the same fibrosarcoma and varying concentrations of recombinant mouse TNF.

**Statistical Analysis**

All data are presented as mean ± SEM. Comparisons among groups were performed using analysis of variance (ANOVA), the Student-Newman-Keuls multiple-range test, χ², and the generalized Wilcoxon-Breslow test.

**RESULTS**

The percentages of pan-T lymphocytes are given in the Table. These differences were statistically significant (P<.0001, ANOVA). The Student-Newman-Keuls multiple-range test revealed that the group receiving transfusion 7 days before endotoxin was grouped with the groups not receiving endotoxin.

The percentages of helper/inducer T lymphocytes among blood mononuclear cells are given in the Table. These differences were statistically significant (P<.0001, ANOVA). The Student-Newman-Keuls multiple-range test demonstrated that the group receiving blood a week before endotoxin was grouped with the non-endotoxin-treated groups.

The percentages of suppressor/cytotoxic T lymphocytes among blood mononuclear cells are given in the Table.
These differences were statistically significant ($P<.0001$, ANOVA). The Student-Newman-Keuls multiple-range test showed that the 12 groups of data fell into two ranges. The first consisted of all groups of rats that did not receive endotoxin except for those rats that received anesthesia 2 days before analysis of lymphocyte subsets. The second consisted of all groups of rats that received endotoxin plus the group that did not receive anesthesia but did receive anesthesia 2 days before lymphocyte analysis.

Blood transfusion administered 7 days before challenge with endotoxin was found to improve survival. The group of rats that received lactated Ringer's solution 7 days before challenge with endotoxin had an 80% mortality. The group receiving anesthesia 7 days before challenge had a 40% mortality, while those rats administered blood transfusions 1 week before challenge had a 0% mortality. These differences were statistically significant ($P<.0001$, $x^2$). The mean survival time in the group given lactated Ringer's solution was 2.20 ± 0.64 days. For the anesthesia-treated group it was 4.87 ± 0.72 days, and for the transfused group, 7.00 ± 0.00 days. These differences were statistically significant ($P<.0001$, generalized Wilcoxon-Breslow test).

Neither blood transfusions nor anesthesia administered 2 days before challenge with endotoxin was found to improve survival. In the control group that received lactated Ringer's solution 2 days before challenge with endotoxin, there was an 86.7% mortality. Those rats receiving anesthesia 2 days before challenge had an 80% mortality, and those receiving blood transfusions 2 days before challenge had a 73.3% mortality. These differences were not statistically significant. The mean survival time in the group given lactated Ringer's solution was 1.80 ± 0.55 days. For the anesthesia-treated group it was 2.60 ± 0.60 days, and for the transfused group, 2.73 ± 0.69 days. These differences did not reach statistical significance.

The TNF level in the plasma of the group given lactated Ringer's solution was 1.14 ± 0.08 x 10^6 U/mL. For the anesthesia-treated group it was 1.40 ± 0.25 x 10^6 U/mL, and for the transfused group, 0.21 ± 0.06 x 10^6 U/mL. These differences were significant ($P = .0004$).

**COMMENT**

Patients suffering traumatic injuries, malnutrition, and sepsis frequently suffer varying degrees of immunosuppression. Such immunodeficiency states can predispose to potentially lethal infectious complications. Patients who undergo surgery may also require anesthesia and blood transfusions. These two therapeutic modalities may also suppress immune function.

Slade et al. demonstrated in normal, healthy, living related renal allograft donors that immune function deteriorated following the administration of anesthesia. This impairment in immune function was broad in nature and became apparent within 10 minutes of the induction of anesthesia.

Blood transfusions have also been demonstrated to impair immune function. This impairment also appears to involve multiple components of the immune system.

We have previously evaluated the effect of blood transfusions and anesthesia on resistance to bacterial infections in animal models. These models demonstrated that blood transfusions or anesthesia administered to healthy Lewis rats before E. coli challenge significantly decreased survival rates. The detrimental effect of transfusions increased with time during the first 7 days following blood administration. In contrast, the effect of anesthesia was most apparent on the day of administration and decreased with time during the first week following anesthesia administration.

Our current study attempted to delineate the exact mechanism of this decreased resistance to E. coli infections. We evaluated the effect of anesthesia and blood transfusions on the host response to E. coli endotoxin. To achieve optimum extrapolation to our previous studies, we chose the same strain of E. coli for our endotoxin model that had been used in our prior studies. The E. coli were heat killed before administration to eliminate any infective component and thereby enable us to evaluate only the endotoxin component.

As in our prior studies, the blood transfusion effect was not apparent at 2 days following transfusion. At 7 days following transfusion, there was a marked transfusion effect demonstrated in several of our models. This included prevention of the endotoxin-induced decrease in the percentage of pan-T lymphocytes and helper/inducer T lymphocytes. In non-endotoxin-challenged rats, blood transfusions failed to alter the percentage of pan-T lymphocytes, helper/inducer T lymphocytes, or suppressor/cytotoxic T lymphocytes.

Since the administration of blood transfusions 7 days before endotoxin challenge improved immune status as measured by percentage of T-lymphocyte populations, it might be expected that it would also improve host resistance to endotoxin challenge. This was indeed found to be the case in our mortality studies. When blood transfusions were administered 7 days before challenge with endotoxin, both the survival rate and the mean survival time increased significantly.

The finding of a transfusion effect in the endotoxin-challenged rats when the transfusion was given 7 days before endotoxin challenge emphasizes two previously established principles of transfusion effects. First, the transfusion effect in both infectious and transplantation models increases with time, at least for the first several days following transfusion. Second, the transfusion effect can be altered by other immunologic manipulations, in this case the administration of endotoxin.

There would appear to be a discrepancy between our current and previous findings in regard to the effect of blood transfusions on immune function. Our previous studies showed that blood transfusions suppress immune function and decrease survival in infectious models. Herein, we have demonstrated improved survival in an endotoxin model and improved immune status as measured by T-cell subsets. This discrepancy may be the result of the fact that in certain clinical conditions, suppression of immune function is beneficial to the host.

Our current study documented an 81% decrease in TNF levels in the transfused rats, which may have been a significant factor in the improved survival rate seen in the rats transfused 7 days before endotoxin challenge, since excessive production of TNF by the host's immune system has been reported to be a major factor in mortality in endotoxin shock. There is additional support for this concept from a report that prostaglandin E decreases production of TNF and increases survival rate in animals challenged with endotoxin, and that blood transfusions increase the rate of synthesis of prostaglandin E.

It would therefore appear possible that the immunosuppressive blood transfusion effect may be detrimental to host resistance to infection while at the same time being beneficial against the endotoxin sequelae of such infections. In those situations where death is more likely to result from the infectious process, transfusions could be expected to increase mortality, while in those situations where the endotoxin component is more likely to result in death, the transfusion might be beneficial to survival rates. Such a possibility may explain the discrepancy in a previously reported study in which transfusions improved survival in certain burned rat sepsis models while decreasing survival rates in other burned rat sepsis models.
References


Discussion

CHRISTOPHER G. BAKER, MD, Chapel Hill, NC: This study demonstrates a decrease in helper/inducer T-cell function along with an 81% reduction in TNF level, which was hypothesized but not proved to be secondary to prostaglandin E₂ production. Which strain of E coli was used? What other functions of T cells (eg, blastogenesis) and macrophage function were measured? How can the differences between the results of this study and former studies be reconciled? What role might monoclonal antibodies have in this model? Anti-prostaglandin? Anti-endotoxin?

MITCHELL FINK, MD, Worcester, Mass: Was the preparation of endotoxin a particulate preparation of killed bacteria or a soluble one to mimic clinical situations. We believed that the possibility of desensitization?

MARK CALLERY, MD, St Louis, Mo: Please speculate on the possible changes in TH-1 and TH-2 cells.

WILLIAM BLAEMORE, Birmingham, LA: Why did you use barbiturate anesthesia, which has been shown to affect cytochrome P-450 and to suppress the immune system?

TIMOTHY PRUETT, Charlottesville, Va: Have you done a dose-response curve with the amount of endotoxin to discriminate lethality differences?

DR WAYMACK: The E coli strain was supplied by Richard Simmons, MD. We are grateful to Christine C. Davis for editorial assistance, Janice L. Duke for library assistance, and John Q. Hester for technical assistance.

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Host Defense—Waymack et al