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Applefeld, Willard
Wang, Jeffrey
Sun, Junfeng
et al.

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In canine bacterial pneumonia circulating granulocyte counts determine outcome from donor cells

Willard N. Applefeld[1], **Jeffrey Wang**[1], **Junfeng Sun**[1], **Steven B. Solomon**[1], **Jing Feng**[1], **Thomas Risoleo**[2], **Irene Cortés-Puch**[3], **Aurélie Gouél-Cheron**[4], **Harvey G. Klein**[5], **Charles Natanson**[1]

[1]Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Maryland

[2]Tufts University, Medford, Massachusetts

[3]Division of Pulmonary, Critical Care and Sleep Medicine, University of California Davis Medical Center, Sacramento, California

[4]Department of Anesthesiology and Intensive Care, Bichat University Hospital, Assistance Publique-Hôpitaux de Paris, Université de Paris, Paris, France

[5]Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland

Abstract

BACKGROUND: In experimental canine septic shock, depressed circulating granulocyte counts were associated with a poor outcome and increasing counts with prophylactic granulocyte colony-stimulating factor (G-CSF) improved outcome. Therapeutic G-CSF, in contrast, did not improve circulating counts or outcome, and therefore investigation was undertaken to determine whether transfusing granulocytes therapeutically would improve outcome.

STUDY DESIGN AND METHODS: Twenty-eight purpose-bred beagles underwent an intrabronchial *Staphylococcus aureus* challenge and 4 hours later were randomly assigned to granulocyte (40–100 × 10^9 cells) or plasma transfusion.

RESULTS: Granulocyte transfusion significantly expanded the low circulating counts for hours compared to septic controls but was not associated with significant mortality benefit (1/14, 7% vs. 2/14, 14%, respectively; p = 0.29). Septic animals with higher granulocyte count at 4 hours (median [interquartile range] of 3.81 3.39–5.05] vs. 1.77 [1.25–2.50]) had significantly increased survival independent of whether they were transfused with granulocytes. In a subgroup analysis,

Address reprint requests to: Willard N. Applefeld, MD, Critical Care Medicine Department, National Institutes of Health, Building 10, Room 2C145, Bethesda, MD 20892; willard. applefeld@nih.gov.

ICP, HGK, and CN conceptualized and designed the study. SS, JF, AGC, and TR performed the experiments. JS performed the statistical formal analysis of the data. WNA, JW, and CN interpreted the data. JF assisted with creation of figures. WNA, JW, and CN wrote the original draft and prepared the manuscript. All authors reviewed the manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

animals with higher circulating granulocyte counts receiving donor granulocytes had worsened lung injury compared to septic controls. Conversely, donor granulocytes decreased lung injury in septic animals with lower counts.

CONCLUSION: During bacterial pneumonia, circulating counts predict the outcome of transfusing granulocytes. With low but normal counts, transfusing granulocytes does not improve survival and injures the lung, whereas for animals with very low counts, but not absolute neutropenia, granulocyte transfusion improves lung function.

During bacterial infection, neutrophils are one of the first lines of host defense.[1–3] Individuals with qualitative or quantitative defects in neutrophil function are more prone to infections.[4–7] Treating neutropenic patients with prophylactic granulocyte colony-stimulating factor (G-CSF) to expand circulating counts and reduce the period of neutropenia reduces fever, hospitalization, number of infections, and antibiotic use[8–14] and lowers all-cause mortality as well as infection-related mortality.[13]

Early observational studies in nonneutropenic septic patients reported significant benefits of therapeutic G-CSF on survival[15–18] and, in some studies, decreased incidence of disseminated intravascular coagulation and acute respiratory distress syndrome.[19] However, randomized clinical trials studying therapeutic G-CSF in non-neutropenic septic subjects yielded equivocal results and no survival benefit. In most studies, the peak neutrophil elevations were not reached until several days after enrollment.[20–25] Therapeutic administration of G-CSF in our canine nonneutropenic model of septic shock, similar to the randomized clinical studies, produced no survival benefit and no acute elevations in neutrophil counts.[26] In contrast, prophylactic G-CSF administration markedly increased granulocyte counts at the onset of infection in canine models of *Escherichia coli* pneumonia and peritonitis[27,28] as well as in rats with *Staphylococcus aureus* pneumonia,[29] and significantly improved survival. We also found that progressively larger, more lethal bacterial challenges, as measured by colonyforming units in our septic shock model, progressively increased mortality and were associated with decreased ability to expand neutrophil counts.[30] Similarly, in clinical sepsis, the inability to expand the neutrophil count early in infection was associated with a poor outcome.[31]

Based on the above results, we hypothesized that the inability of therapeutic G-CSF to acutely expand neutrophil counts in nonneutropenic septic subjects, might explain its lack of benefit. We wondered whether the beneficial effect of prophylactic G-CSF therapy was related to the expanded neutrophil count at the onset of infection which might offer some degree of early protection. Transfusing donor granulocyte concentrates instead of administering therapeutic G-CSF to acutely increase circulating neutrophils at the onset of infection could circumvent this problem. Donor granulocyte transfusions have a long history of usage in clinical practice, but their use has been traditionally limited to treating severe infections in patients with preexisting neutropenia or neutrophil dysfunction. However, this approach has not yet been evaluated as an adjunctive therapy for immunocompetent patients with severe infections or sepsis. We designed a study to investigate whether administration of functional neutrophils in the form of donor granulocyte transfusions might serve as effective adjunctive therapy for sepsis. To determine the potential benefit of donor

neutrophils, we challenged animals intrabronchially with a dose of bacteria known to be associated with a decrease in circulating neutrophil counts and poor survival.

MATERIALS AND METHODS

Twenty-eight purpose-bred beagles (18–30 months, 10–13 kg, male, Covance Inc.) were studied. Each study week (cycle), 2 of the 28 animals were anesthetized, intubated, and mechanically ventilated. Central venous, femoral arterial, and indwelling urinary catheters and a tracheostomy were placed in the two study animals each week, as previously described.[32] On Day 1 at Time 0 hour each study cycle, the two study animals received an intrabronchial challenge of *S. aureus* ($0.5–2 \times 10^9$ CFU/kg) and were followed for 5 days (96 hr) as previously described.[32] Granulocyte concentrates ($40–100 \times 10^9$ cells) or an equivalent volume of fresh frozen plasma (ABRINT) were transfused starting 4 hours after bacterial challenge. To account for any potential effect of host underlying granulocyte level response to early sepsis, septic animals were randomly assigned based on that response. At 4 hours after bacterial inoculation, the animal with the higher granulocyte level was randomly assigned to receive the granulocyte infusion versus plasma, whereas the lower-count animal received the other product.

All animals received antibiotic therapy (ceftriaxone 50 mg/kg) starting at 4 hours after bacterial challenge (starting time of granulocyte transfusion) and then once daily for the duration of the experiment. To simulate standard human septic shock care, fluids and vasopressor support, mechanical ventilation, sedation, and analgesia were given throughout the 96-hour study based on standardized protocols in which care is titrated to physiologic endpoints as previously described.[32] Standard veterinarian intensive care measures were also performed during this time including prophylaxis for pressure ulcers (rotation every 24 hr), gastric stress ulcer prevention (famotidine 10 mg IV every 12 hr), pulmonary embolism and deep venous thrombosis prophylaxis (heparin subcutaneously 3000 IU every 8 hr) as previously described.[32] All animals were treated identically, except for the experimental intervention.

Animals were continuously monitored and cared for by a clinician or trained technician throughout the experiment. At standard time points, blood, urine, and sputum were collected for further analysis. After 96 hours, animals still alive were considered survivors and were euthanized. The study protocol was reviewed and approved by the National Institutes of Health Clinical Center Institutional Animal Care and Use Committee (CCM18–03). For statistical and granulocyte collection methods, please see e-supplementary methods, available as supporting information in the Online Supplement to this paper.

RESULTS

Peripheral Counts after granulocyte transfusion

At 4 hours after bacterial challenge, immediately before granulocyte transfusion therapy (baseline), there was no significant difference in mean peripheral granulocyte counts in septic animals randomly assigned to receive granulocyte transfusions versus septic controls randomly assigned to receive an equivalent volume of plasma ($p = 0.80$). The mean

(standard error [SE]) number of granulocytes (in billions) collected for transfusion from donors during the 14 study cycles was 60.5 (19.4). For the mean (SE) number of other cell products collected as bystanders (lymphocytes, monocytes, platelets, hemoglobin), please see Table S1 (available as supporting information in the online version of this paper). In the 14 septic animals transfused granulocytes, there was a significantly greater increase in the mean peripheral granulocyte counts from baseline to 6 hours ($p = 0.03$) and 8 hours ($p = 0.03$) compared to that of the 14 septic controls (Fig. 1A). There were no other significant differences in mean granulocyte counts between the two groups. Our aim was to collect and transfuse greater than 50×10^9 donor granulocytes; we therefore also examined the subset of animals transfused greater than 50×10^9 donor granulocytes. The 9 animals receiving greater than 50×10^9 granulocytes had a significantly greater increase from baseline in mean peripheral granulocyte count at 6, 8, and 10 hours after bacterial inoculation in comparison to their 9 matched septic controls ($p < 0.0001$, $p = 0.003$, and $p = 0.03$, respectively) (Fig. 1B). There were no other significant differences in circulating granulocytes in that subset of animals throughout. The 5 animals transfused with fewer than 50×10^9 granulocytes in comparison to the 5 time-matched septic controls had no significantly different change from baseline in mean peripheral granulocyte counts throughout the study (Fig. 1C). Examining only the 14 septic animals receiving donor granulocytes, we found a strong and significant positive correlation between the number of granulocytes transfused and the increase in the number of peripheral granulocytes counts from baseline to 6 hours ($r = 0.64$, $p = 0.01$), 8 hours ($r = 0.65$, $p = 0.01$), and 10 hours ($r = 0.54$, $p = 0.05$) after bacterial challenge (Fig. 2).

Peripheral Counts of other cells transfused

At 4 hours after bacterial challenge, immediately before granulocyte transfusion (baseline), there were no significant differences in mean peripheral monocyte, lymphocyte, and platelet counts in septic animals randomized to receive granulocyte transfusions versus septic controls (data not shown, $p > 0.05$). In the 14 septic animals transfused granulocytes, there was a significantly greater increase from baseline in the mean peripheral lymphocyte counts at 6 hours ($p = 0.002$), 10 hours ($p = 0.008$), and 12 hours ($p = 0.03$); platelet counts at all time points measured from 6 to 40 hours ($p = 0.02$ to $p < 0.0001$); and hemoglobin levels at 6, 8, 10, 32, 40, and 48 hours ($p = 0.05$ to $p < 0.0001$) compared to the 14 septic controls receiving over the same time period (Figs. S1–S1A, available as supporting information in the online version of this paper). The 9 animals receiving greater than 50×10^9 granulocytes had a significantly higher change from baseline in the mean peripheral monocyte counts at 6 hours ($p = 0.03$) and 10 hours ($p = 0.02$); mean lymphocyte counts at 6 hours ($p < 0.0001$), 10 hours ($p = 0.002$), 12 hours ($p = 0.001$), and 16 hours ($p = 0.02$); platelet counts at all time points measured from 8 to 24 hours ($p = 0.03$ to $p < 0.0001$); and mean hemoglobin levels at 6 hours ($p = 0.01$), 10 hours ($p = 0.04$), 24 hours ($p = 0.04$), and 40 hours ($p = 0.01$) after bacterial inoculation in comparison to their 9 matched septic controls (Figs. S1–S1B, available as supporting information in the online version of this paper). The 5 animals transfused with fewer than 50×10^9 granulocytes in comparison to the 5 time-matched septic controls receiving plasma had significantly higher change from baseline in mean hemoglobin levels at 10 hours ($p = 0.03$), 32 hours ($p = 0.02$), and 40 hours ($p = 0.02$), and platelet levels at all time points measured from 6 to 48 hours ($p = 0.006$ to $p < 0.0001$) but no significant change from baseline in mean peripheral monocyte counts,

and a significantly lower increase from baseline in mean lymphocyte counts at 40 hours ($p = 0.04$) and 48 hours ($p < 0.0001$) after bacterial inoculation in comparison to their 5 matched septic controls (Figs. S1–S1C, available as supporting information in the online version of this paper).

Mortality

The 14 septic animals receiving donor granulocytes did not have a significantly improved survival when compared to the 14 septic control animals transfused with an equal volume of plasma (1/14, 7% vs. 2/14, 14%, respectively; $p = 0.29$; stratified log-rank test; Fig. 3A). We also did not observe a significant survival benefit looking at only the subset of 9 septic animals receiving greater than 50 billion granulocytes in comparison to the 9 time-matched septic controls receiving plasma (1/9, 11% vs. 2/9, 22%, respectively; $p = 0.32$; stratified log-rank test; Fig. 3B). Blood cultures performed daily during the study period were negative for growth of bacteria in all animals studied.

Peripheral granulocyte Counts 4 hours after bacterial challenge and survival

To prevent potential confounding of the baseline peripheral granulocyte count to the treatment effect of donor granulocyte infusion, each week the randomization of the two animals was stratified with respect to the granulocyte count at 4 hours (i.e., higher vs. lower). As expected by design, over the 14 weeks of the study, the 14 septic animals selected at 4 hours with higher granulocyte counts had overall higher granulocyte counts (median [interquartile range (IQR)], 3.81 [3.39–5.05]) than those 14 septic animals with lower granulocyte counts at 4 hours (median [IQR] of 1.77 [1.25, 2.50]) (see Fig. 4 for individual values by group). Unexpectedly, independent of the granulocyte transfusion, the 14 septic animals with the higher granulocyte count over the 14 weeks had overall a significantly improved survival compared to the 14 animals with the lower granulocyte count at 4 hours ($p = 0.03$; stratified log-rank test) (Fig. 5). Over the 14 study cycles of two animals per cycle, for 11 of 14 cycles the higher baseline granulocyte count animal survived longer. In 3 of the 14 weeks, the lower-count animal survived longer (Fig. 5).

Lung injury score

We used a previously described lung injury score (LIS) to increase our ability to quantify the extent of lung damage.[33] Briefly, the LIS is a composite marker of mean pulmonary artery pressure, alveolar oxygen gradient, plateau pressure measurement, peripheral capillary oxygen saturation, and respiratory rate with higher scores indicating greater lung injury. The effect of donor granulocytes on mean LIS was statistically significantly different and opposite at 12, 16, 32, and 40 hours after bacterial inoculation depending on the whether the septic animal had a high or low peripheral granulocyte count at baseline ($p = 0.05$ to <0.0001 for interaction) (Fig. 6) (for individual components of the LIS, please see Figs. S5–S9, available as supporting information in the online version of this paper). At 12, 16, 32, and 40 hours after bacterial inoculation, septic animals with a high baseline granulocyte count had an increase (worsening) in mean LIS with transfusion of donor granulocytes compared to those with a high peripheral count receiving plasma. Transfusion of donor granulocytes in septic animals with a low baseline peripheral granulocyte count resulted in an improvement in mean LIS compared to those with a low peripheral count receiving

plasma only. For animals receiving plasma, having a higher peripheral granulocyte count at 4 hours was associated with less lung injury and a lower (improved) mean LIS. At 4 hours, lower granulocyte counts were associated with a worsened mean LIS score. When exogenous granulocytes were transfused, this relationship between peripheral granulocyte counts and LIS reversed; transfusing granulocytes to animal with relatively high peripheral counts increased (worsened) LIS, and administering granulocytes to animal with relatively low granulocyte counts lowered (improved) LIS.

Lactate

The septic animals described above receiving donor granulocytes with increased mean LIS at 12, 16, 32, and 40 hours and a high baseline granulocyte count also had a greater increase from baseline in mean lactate level at 32 ($p = 0.055$), 40 ($p = 0.003$), and 48 hours ($p = 0.04$) compared to septic animal receiving plasma (Fig. 7). In addition, at 32 hours, similar to LIS, the effect of donor granulocytes on mean lactate levels was statistically significant and opposite depending on the whether the septic animal had a high or low peripheral granulocyte count at baseline ($p = 0.02$ for interaction). At 32 hours, septic animals with a high baseline granulocyte count had an increase in mean lactate levels with transfusion of donor granulocytes, whereas transfusion of donor granulocytes in septic animals with a low baseline peripheral granulocyte count had an improvement (lowering) in mean lactate levels compared to septic animals receiving plasma transfusion. The overall findings of lactate mirrored the LIS findings.

Shock score/cardiac output and filling pressures (central venous pressure and pulmonary artery occlusion pressure)

The shock score incorporates the level of vasopressor support with norepinephrine to maintain mean arterial blood pressure at a preset normal level for canines (mean 80 mm Hg). We found no significant difference in the degree of hemodynamic derangement between septic animals transfused granulocytes or transfused plasma as measured by the mean change from baseline in shock score as sepsis progressed (all time points, $p > 0.05$) (Fig. S10, available as supporting information in the online version of this paper). We also found no significant difference throughout the study in the degree of hemodynamic derangement as measured by the mean change from baseline in cardiac output, central venous filling pressure, and pulmonary artery occlusion pressure) between septic animals transfused granulocytes or transfused plasma (all time points, $p > 0.05$) (Fig. S11, available as supporting information in the online version of this paper).

Acid Base, renal function, electrolyte status, glucose, hepatic function

There were isolated significant differences in acid base (pH, arterial partial pressure of CO_2, serum bicarbonate concentration, and base excess), renal function (creatinine, blood urea nitrogen), electrolytes (sodium, potassium, chloride, and glucose), and liver function (aspartate aminotransferase, alanine aminotransferase, total bilirubin, lactic acid dehydrogenase, and albumin) at individual time points. However, there were no consistent significant findings over time, which would suggest a meaningful difference between these parameters in the two study groups (Tables S2 through S5).

Cytokine levels

We found that in animals receiving granulocytes versus plasma, there were no significant differences in the mean (±SE) changes from 4 hours (baseline) to 6 hours and 12 hours in cytokine levels (log_{10} [interleukin (IL)-6] in the animals receiving granulocytes at 4 hr, 2.44 ± 0 .22; 6 hr, 3.33 ± 0.22; 12 hr, 3.61 ±.23 vs.animals receiving plasma at 4 hr, 2.56 ± 0.22; 6 hr, 3.45 ± 0.22; 12 hr, 3.94 ± 0.22 [p = 0.27]; log_{10} [IL-10] in animals receiving granulocytes at 4 hr, 1.68 ± 0.24; 6 hr, 0.99 ± 0.24; 12 hr, 1.49 ± 0.25 vs. animals receiving plasma at 4 hr, 1.39 ± 0.24; 6 hr, 1.08 ± 0.24; 12 hr, 2.10 ± 0.24 [p = 0.50]; log_{10} [tumor necrosis factor (TNF)] in animals receiving granulocytes at 4 hr, 0.75 ± 0.24; 6 hr, 0.84 ± 0.24; 12 hr 0.97 ± 0.25 vs. animals receiving plasma at 4 hr, 0.33 ± 0.24; 6 hr, 0.50 ± 0.24; 12 hr, 1.07 ± 0.24 [p = 0.12]). Averaging over all animals (granulocyte transfused and plasma transfused), we found a statistically significant increase in log_{10} (IL-6) from 4-hour baseline (2.50 ± 0.18) to both 6 and 12 hours (3.39 ± 0.16, 3.76 ± 0.14; $p < 0.0001$ for both). We found a statistically significant decrease in log_{10} (IL-10) from 4-hour baseline (1.54 ± 0.18) to 6 hours (1.04 ± 0.06; p = 0.03) and a statistically significant increase in log_{10} (TNFα) from 4-hour baseline (0.54 ± 0.18) to 12 hours (1.01 ± 0.17 p = 0.007).

DISCUSSION

We investigated whether transfusion of donor granulocytes would serve as effective adjunctive therapy in a severe *S. aureus* bacterial pneumonia model of septic shock. In these animals challenged intrabronchially with *S. aureus*, we found that transfusing substantial numbers of donor granulocytes (mean > 60 billion) significantly increased circulating counts over hours but did not significantly improve survival. However, the absolute number of circulating endogenous granulocytes at the onset of infection (time t = 4 hr) was an important prognostic factor during septic shock. Independent of donor granulocyte therapy, septic animals with a higher endogenous circulating granulocyte count (median [IQR], 3.81 [3.39–5.05] vs. 1.77 [1.25–2.50]) early on during sepsis at the time of randomization had increased survival.

In certain subgroups, transfusing exogenous granulocytes actually led to harm. Specifically, animals with normal circulating granulocyte counts at the onset of infection that received exogenous donor granulocytes actually had higher LIS (worsened lung injury) when compared to septic animals that received a matched volume of plasma. In contrast, animals with very low endogenous circulating granulocyte counts transfused exogenous donor granulocytes compared to septic animals receiving plasma had decreased LIS (improved lung injury). These data indicate that animals able to maintain normal peripheral endogenous granulocyte counts early on in septic shock did not benefit from transfusion of donor granulocytes; more concerning is that this procedure may even be harmful in this population. Conversely, subjects with septic shock accompanied by acute severely decreased endogenous peripheral granulocyte counts, although not necessarily at neutropenic levels, may derive clinical benefit during pneumonia with transfusion of donor granulocytes.

Granulocytes reportedly migrate from the vasculature to the lungs within 1 to 4 hours after transfusion.[34] Furthermore, in infected neutropenic human patients, donor granulocytes also traffic to the inflammatory lesion.[34,35] Thus, transfused donor granulocytes likely

traffic to the lungs in the studied septic animals where there was already an inflammatory lesion. In this model of bacterial pneumonia, we hypothesize that the marked trafficking to the lung of donor granulocytes in animals with normal circulating granulocyte counts may have significantly increased the local inflammatory process to the point that further harm was produced. The increased generation of toxic granules augmented release of antimicrobial peptides, and further creation of reactive oxygen species by the increased number of granulocytes in this area, and neutrophil extracellular traps[36] could have potentiated collateral damage to tissues and worsened lung injury. In contrast, in the animals in which sepsis had induced an abnormally low circulating granulocyte count, transfusion of exogenous granulocytes may have replaced deficient native granulocyte defenses and resulted in protection from lung injury by the offending microorganism. Our data support the notion that although granulocytes play a key role in host defense by trafficking to sites of injury and exerting their antimicrobial effects, in animals with normal endogenous granulocyte counts this local amplification can be detrimental and result in collateral damage to the lung. Our data suggest that studies of patients with pneumonia and sepsis-induced abnormally low granulocyte counts may be warranted, even if these patients do not meet criteria for absolute neutropenia.

Our hypothesis that granulocytes and lymphocytes localize to the lung is supported by the sustained levels in the other cell lines detectable in the peripheral blood over the course of the study. In addition to granulocytes, red blood cells (RBCs), monocytes, and platelets are invariably found in granulocyte concentrates collected by leukapheresis and are transfused. During the 5-day (96-hr) study, levels of RBCs, monocytes, and platelets remained elevated, indicating that these cells remained in the intravascular space. This contrasts with the circulating granulocytes and lymphocytes, the levels of which rise quickly in the peripheral circulation in response to transfusion, then fall, indicating that they do not remain intravascular but instead either move out of the bloodstream and localize to the site of infection or are destroyed. These data and the published literature taken together support our hypothesis that the donor granulocytes travel to the infectious sites in the lungs and produce harm or benefit depending on the baseline endogenous granulocyte counts.

There have been no clinical studies examining the effects of therapeutic granulocyte transfusion in nonneutropenic subjects with sepsis. Sauer et al. described first-in-sepsis animal models of an extracorporeal circuit incorporating granulocytes, which improved survival[37,38]; however, they found it only lowered vasopressor requirements when applied to septic nonneutropenic humans but afforded no mortality benefit.[39] Therapeutic granulocyte transfusions in the setting of severe neutropenia (absolute neutrophil count $<0.5 \times 10^9$) are used clinically to treat patients with documented infections unresponsive to antimicrobial therapy as a bridge to bone marrow recovery.[40] This practice is supported by multiple clinical trials from the 1970s in which transfusion of donor granulocytes imparted a survival benefit in neutropenic patients with evidence of or concern for infection.[41–45] Later clinical studies have been unable to confirm these promising results but used suboptimal numbers of granulocytes and were also underpowered.[46–51] Similarly, human studies of transfusion of prophylactic granulocyte in noninfected neutropenic human subjects have had mixed results with some studies showing improvement in survival and rates of infection,[52–56] while others showed no benefits but also no increased mortality with the practice.[57–59] More recently,

therapeutic granulocyte transfusion has been explored in states of granulocyte dysfunction such as systemic fusariosis[60] and chronic granulomatous disease with improvement in clinical response rate.[61,62] Transfusion of therapeutic granulocytes also has been shown to have survival benefits in studies of children and infants with neutropenia induced by either sepsis or chemotherapy,[63–68] while other studies found no benefit but also no evidence of harm.[69–73] There is also evidence in neutropenic septic canine models that granulocyte transfusions improve mortality,[74–78] shorten time to clearance of the offending microorganism from the bloodstream and tissues,[79–82] and decrease the risks of developing infection.[83] Our data are the first to evaluate therapeutic granulocyte transfusions in nonneutropenic subjects unable to expand circulating counts challenged with a severe infectious stimulus.

There were some limitations to our study. First, multiple doses or higher single doses of donor granulocytes might have been required to see a mortality difference between the two groups. Second, we only studied canines infected with intrabronchial *S. aureus*. A different model of sepsis, a different microbial organism, or a different lethality challenge in this model might have resulted in a different effect.

While there have been many studies in neutropenic adults, pediatric and neonatal patients, and neutropenic animals, to our knowledge our study is the first randomized trial to utilize therapeutic granulocyte transfusions in the treatment of sepsis in nonneutropenic hosts with failure to expand the circulating granulocyte counts. There has been historical concern that transfused granulocytes might cause lung injury, with a variety of different trials in neutropenic human adults, pediatric patients, and animals reporting adverse pulmonary effects that ranged from minor and sporadic to substantial.[49,51,53–55,69,84,85] Our study confirms this possibility and suggests that the effect of transfusing exogenous granulocytes is complicated and depends on the baseline endogenous granulocyte count in the host. Transfusion of exogenous granulocytes may increase lung injury during pneumonia with relatively preserved peripheral granulocyte counts. However, during bacterial pneumonia with profoundly low granulocyte counts, although not at the neutropenic level, transfusion of exogenous granulocytes may offer protection from lung injury. Extending these findings to clinical practice suggests that the endogenous circulating granulocyte count may have a profound impact on outcome and on the impact of donor granulocyte transfusions in settings beyond severe neutropenia. Subjects with pneumonia who are septic and have low but still normal peripheral granulocyte counts would not benefit and may even be harmed by transfusion of donor granulocytes. Conversely, our study suggests a possible benefit of donor granulocytes to limit lung injury during bacterial pneumonia associated with profoundly low circulating granulocyte counts induced by sepsis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS:

G-CSF	granulocyte colony-stimulating factor
IL	interleukin
LIS	lung injury score

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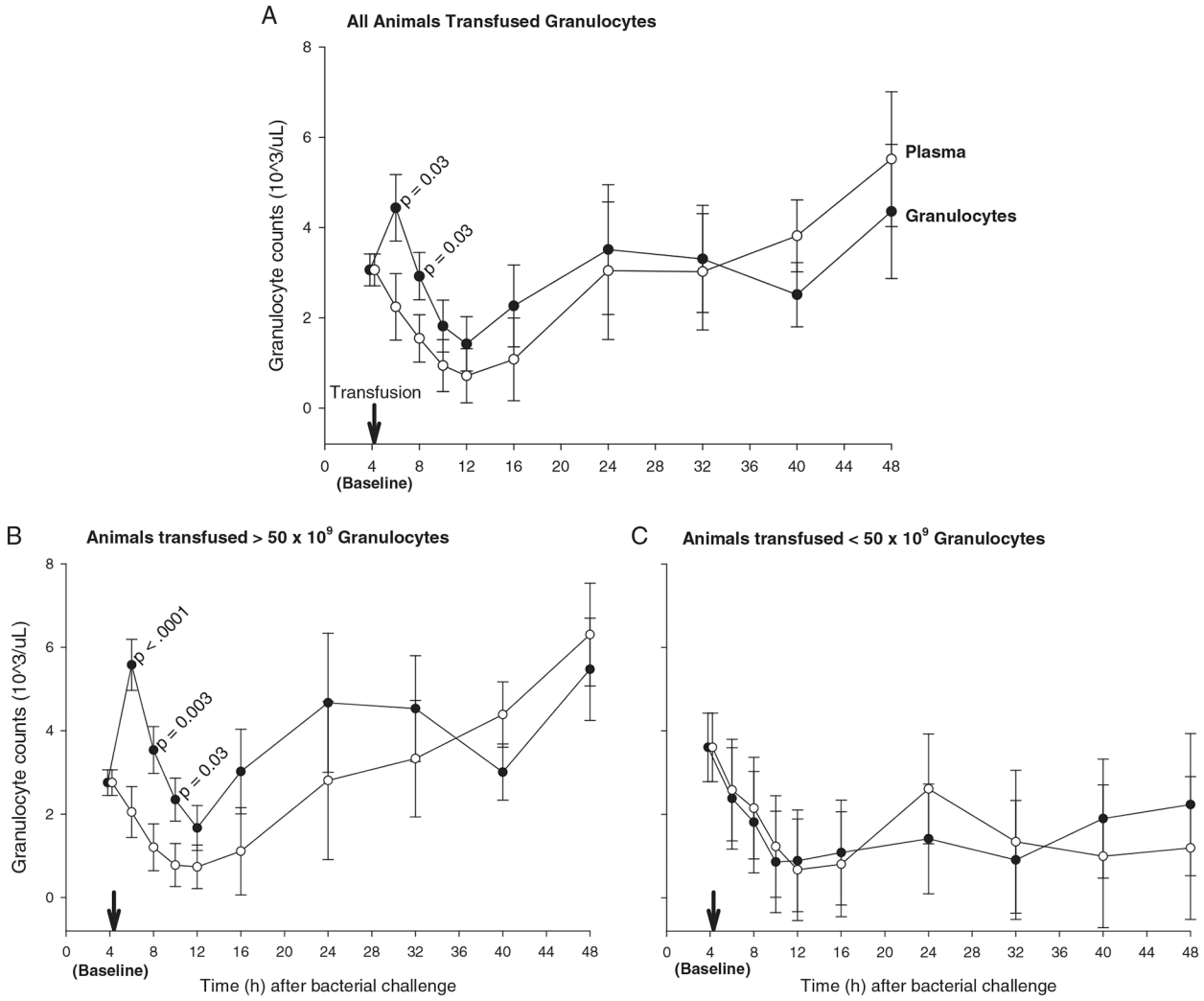

Fig. 1.

Changes in mean (±SE) peripheral granulocyte counts over time. At time t = 4 hours transfusion of exogenous donor granulocytes or matched volume of plasma was started. (A) Comparison of mean peripheral granulocyte counts at select time points in all canines that received transfusion of donor granulocytes versus matched volume of plasma. (B) Comparison of mean peripheral granulocyte counts at select time points in canines that received transfusion of >50 × 10^9 donor granulocytes versus matched volume of plasma. (C) Comparison of mean peripheral granulocyte counts at select time points in canines that received transfusion <50 × 10^9 donor granulocytes versus matched volume of plasma. Statistically significant differences in the mean peripheral granulocyte counts were noted at time t = 6 hours, 8 hours in Fig. 1A and at time t = 6, 8, and 10 hours in Fig. 1B.

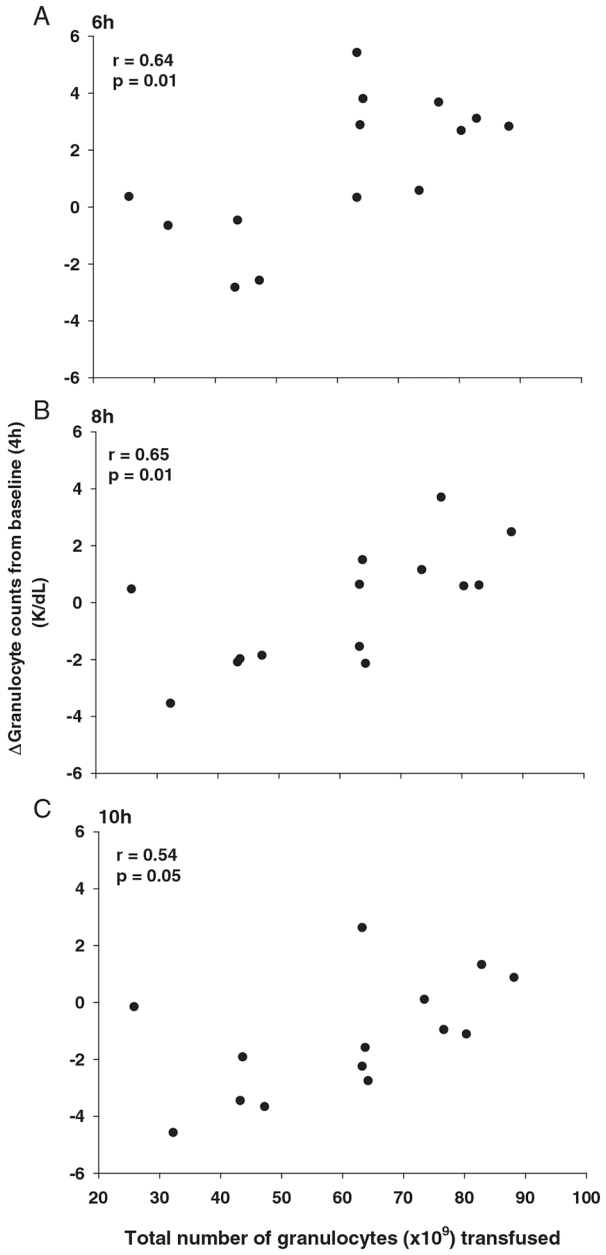

Fig. 2.

The correlation between number of donor granulocytes transfused (× 10^9) versus the change in peripheral granulocyte count from baseline. Correlation figures shown at (A) time t = 6 hours, (B) time t = 8 hours, and (C) time t = 10 hours. Strong positive correlation between number of granulocytes transfused and change in the peripheral granulocyte count from baseline at t = 6 hours and t = 8 hours and a moderate positive correlation at t = 10 hours. We used Pearson product–moment correlation, and the p values were calculated using t tests.

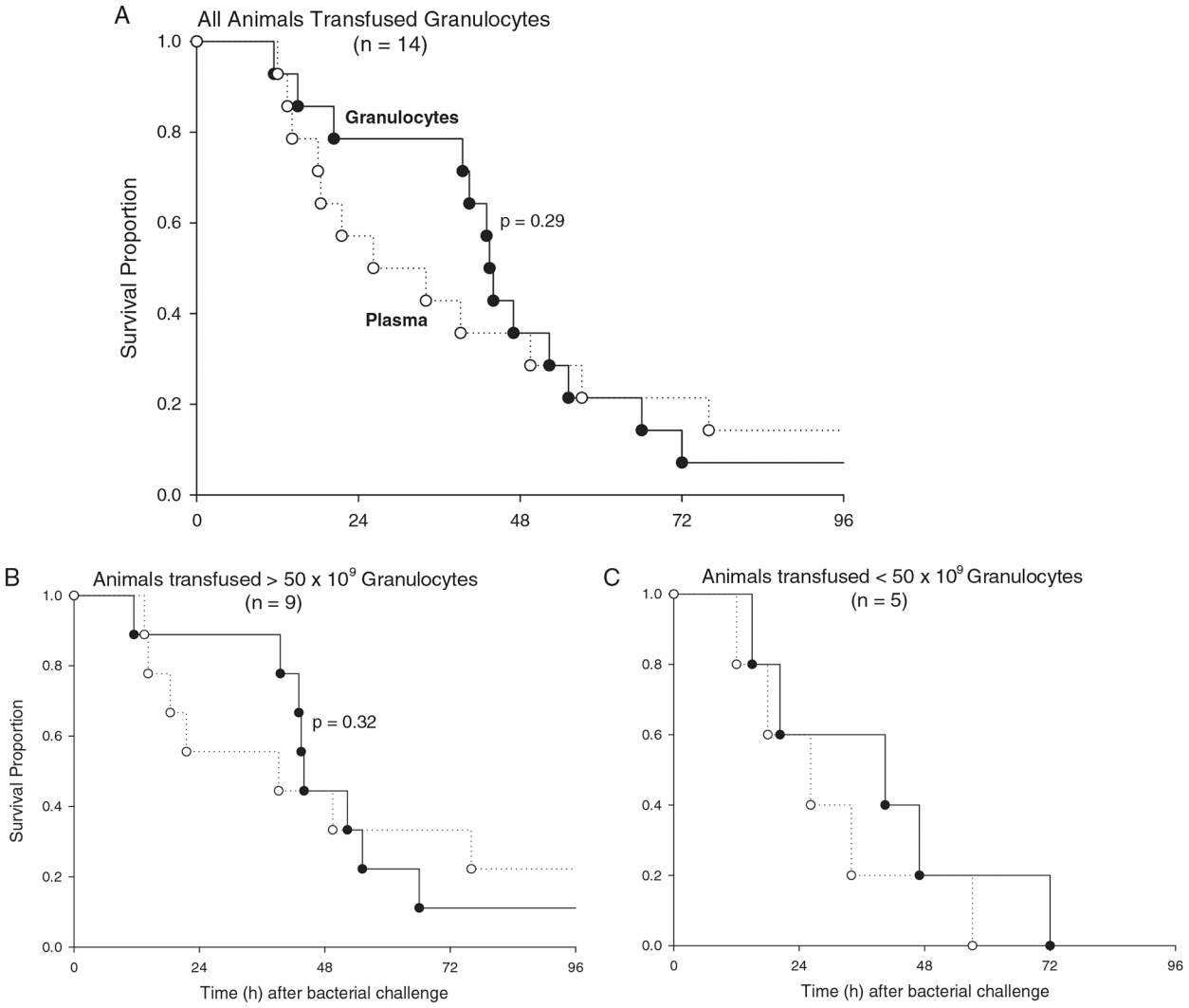

Fig. 3.

Kaplan–Meier curves comparing the proportion of animals surviving that received transfusion of donor granulocytes versus matched volume of plasma. There was no statistically significant difference in survival between (A) all animals transfused donor granulocytes versus matched volume of plasma using stratified log rank tests. In the subset of animals that received (B) >50 × 10^9 donor granulocytes versus matched volume of plasma, there was no significant difference in survival. In the subset of animals that received <50 × 10^9 donor granulocytes versus matched volume of plasma (C), there was no statistically significant difference in survival (stratified log-rank tests).

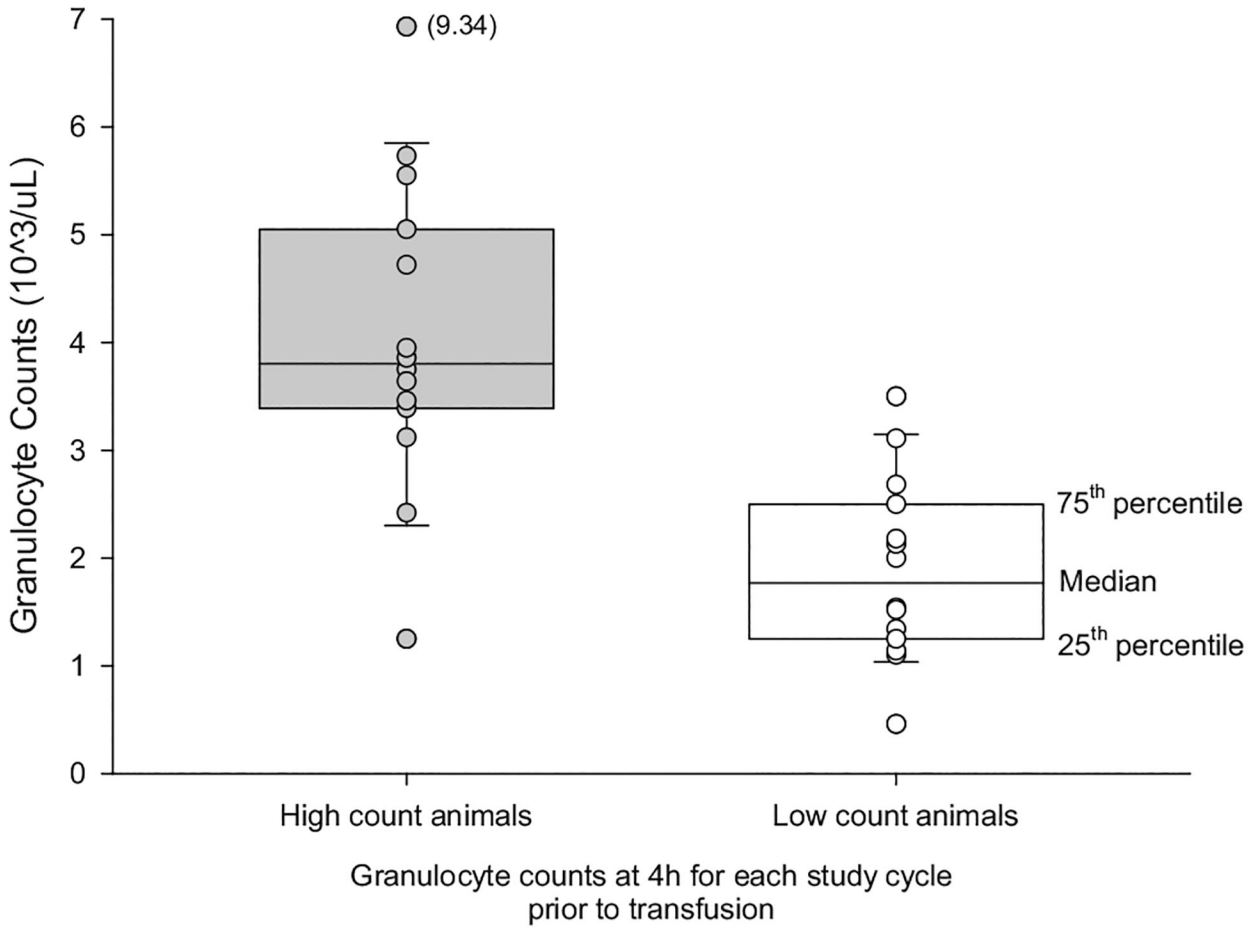

Fig. 4.

Baseline endogenous granulocyte counts at time t = 4 hours after infectious challenge, immediately before transfusion of exogenous granulocytes. As expected by design, there exists a significant difference in the granulocyte counts between animals in the high baseline granulocyte count group versus low baseline granulocyte count group.

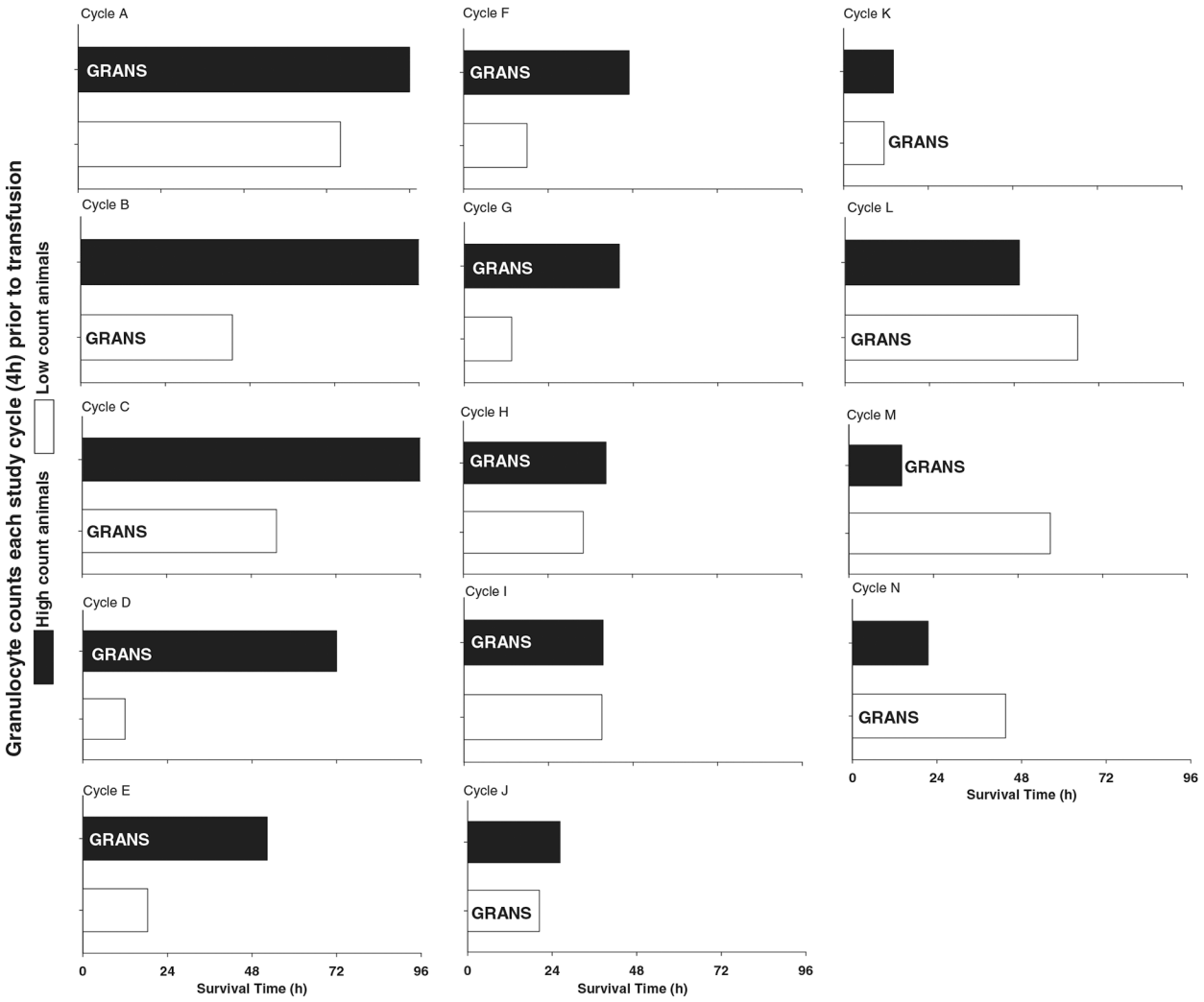

Fig. 5.

Survival time for animals in each of the 14 cycles. At time t = 4 hours after infectious challenge, immediately before donor granulocyte transfusion, animals were subdivided into high and low count groups based on which animal had the higher and lower granulocyte count at that time. Animals each week with the higher baseline granulocyte count are designated by black bars. Animals with the lower baseline granulocyte count are designated by white bars. By design, animals were randomized to receive either transfusion of donor granulocytes or transfusion of a matched volume of plasma, such that the group (high vs. low) being transfused with donor granulocytes varied cycle to cycle. Transfusion of granulocytes is denoted by label “GRANS.” Survival time was significantly longer in animals with a higher granulocyte count at baseline in 11 of 14 cycles.

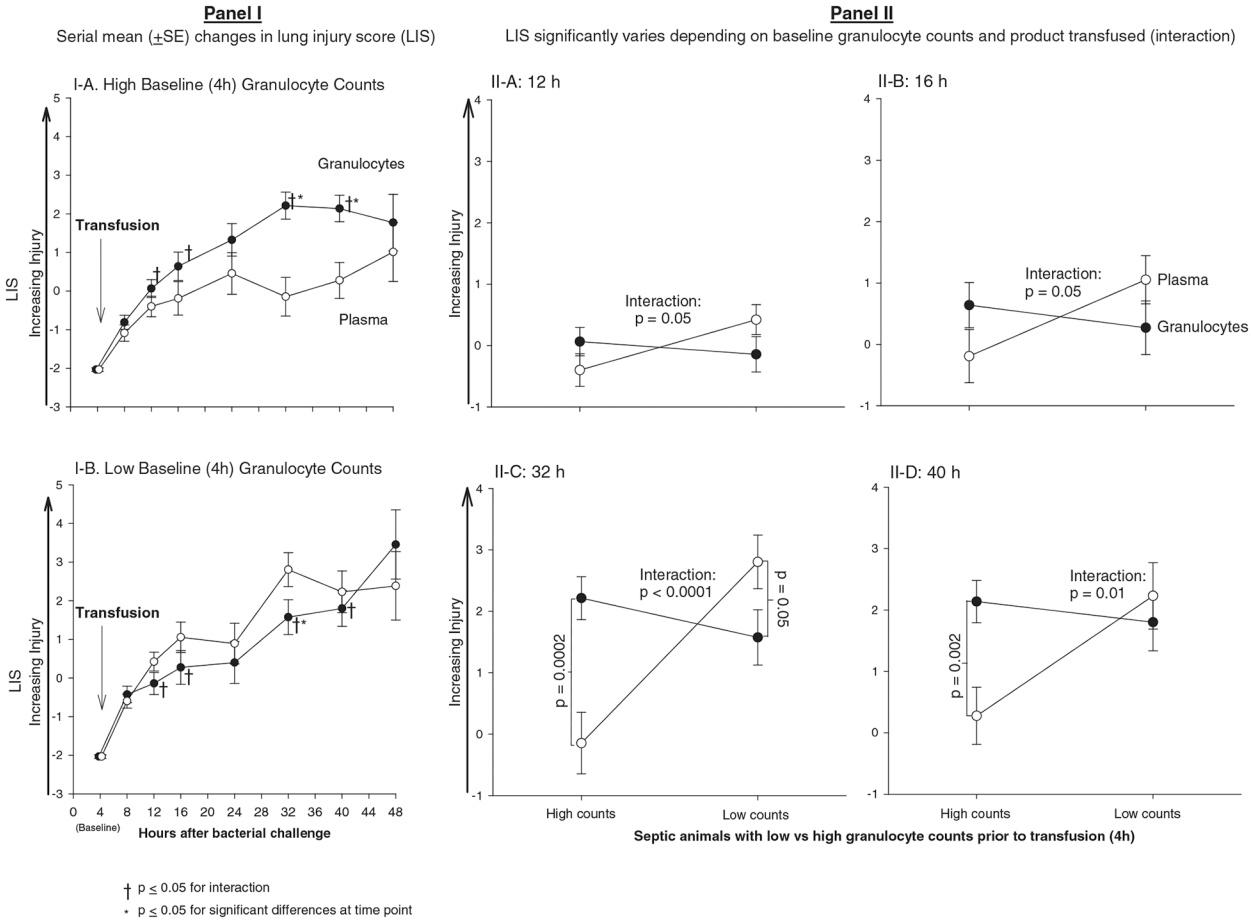

Fig. 6.

Panel I: Changes in mean (±SE) lung injury score (LIS) counts over time comparing (I-A) animals with a high baseline granulocyte count at time t = 4 hours that received donor granulocyte transfusion versus matched volume of plasma. (I-B) Animals with low baseline granulocyte count at time t = 4 hours that received donor granulocyte transfusion versus matched volume of plasma. Panel II: Interaction figures demonstrating the opposite effect of donor granulocyte transfusion on LIS depending on baseline (t = 4 hr) circulating granulocyte count and if the septic animal received donor granulocytes or plasma. Statistically significant qualitative interactions are seen at time t = 12, 16, 32, and 40 hours as follows: At each of these time points, granulocyte transfusions correlated to higher lung injury scores in animals with high baseline granulocyte counts, whereas granulocyte infusions correlate to lower lung injury scores in animals with low baseline granulocyte counts. The opposite is true for septic animals receiving plasma. Plasma transfusions correlated to lower LIS in animals with high baseline granulocyte counts. Plasma infusions correlate to higher LIS in animals with low baseline granulocyte counts.

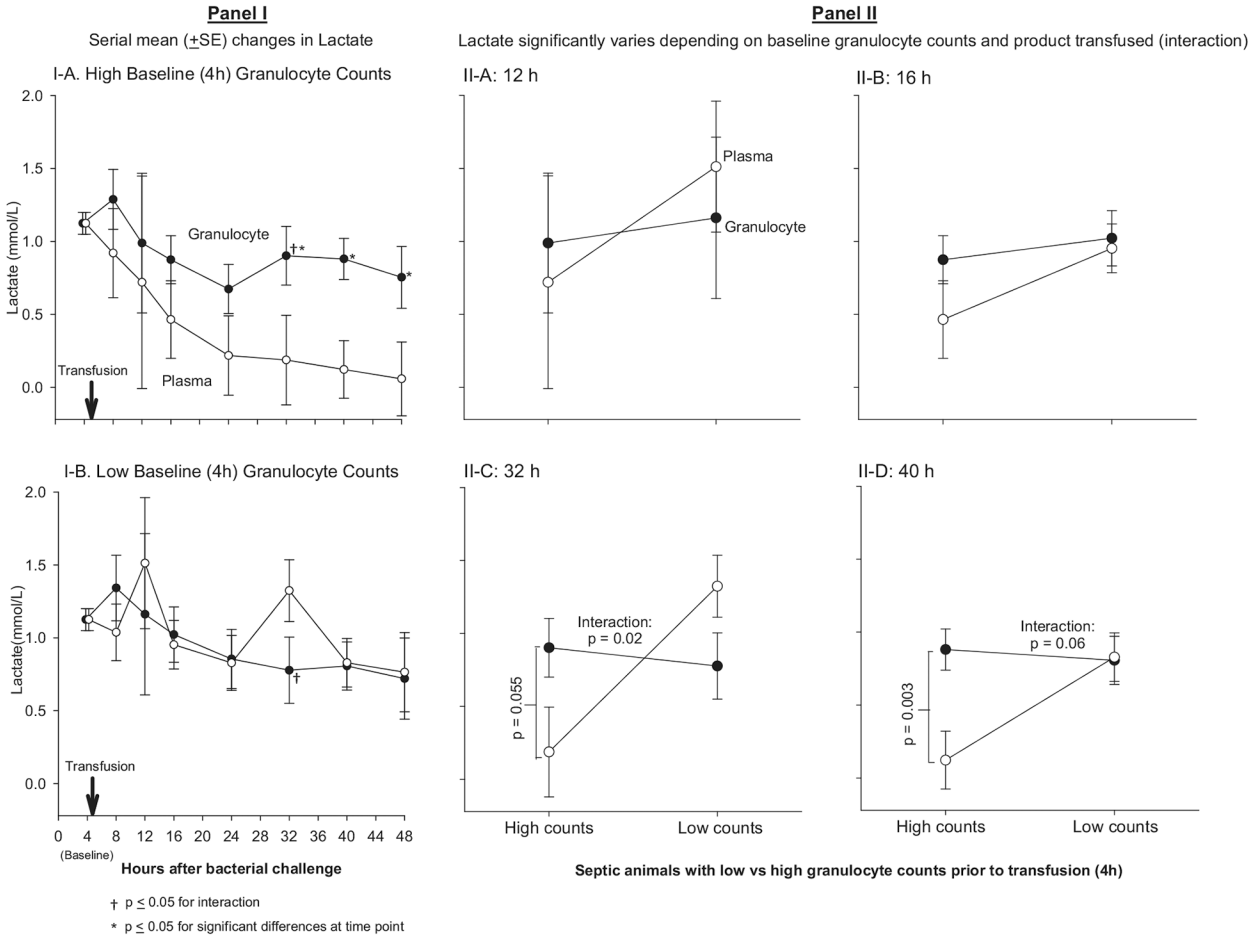

Fig. 7.

Panel I: Changes in mean (±SE) lactate (mmol/L) over time comparing (I-A) animals with a high baseline granulocyte count at time t = 4 hours that received donor granulocyte transfusion versus matched volume of plasma. (I-B) Animals with low baseline granulocyte count at time t = 4 hours that received donor granulocyte transfusion versus matched volume of plasma. Panel II: Interaction figures demonstrating the opposite effect of donor granulocyte transfusion on lactate depending on baseline granulocyte count and if the septic animal received donor granulocytes or plasma. Data consistent with qualitative interactions are seen at time t = 12 hours and 40 hours, and a statistically significant interaction at t = 32 hours. Donor granulocyte transfusions correlated to higher lactates in animals with high baseline granulocyte counts. Granulocyte infusions correlated to lower lactates in animals with low baseline granulocyte counts. The opposite was true for animals that received plasma instead of granulocyte infusion. Plasma transfusions correlated to lower serum lactates in animals with high baseline granulocyte counts. Plasma infusions correlate to higher serum lactates in animals with low baseline granulocyte counts.