Blood Transfusion Promotes Cancer Progression: A Critical Role for Aged Erythrocytes

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Abstract

Background—In cancer patients, allogeneic blood transfusion is associated with poorer prognosis, but the independent effect of the transfusion is controversial. Moreover, mediating mechanisms underlying the alleged cancer promoting effects of blood transfusion are unknown, including the involvement of donors' leukocytes, erythrocytes, and soluble factors.

Method—Two syngeneic tumor models were used in Fischer 344 rats, the MADB106 mammary adenocarcinoma and the CRNK-16 leukemia. Outcomes included host ability to clear circulating cancer cells, and host survival rates. The independent impact of blood transfusion was assessed, and potential deleterious characteristics of the transfusion were studied, including blood storage duration, the role of erythrocytes, leukocyte, and soluble factors, and the kinetics of the effects.

Results—Blood transfusion was found to be an independent and significant risk factor for cancer progression in both models, causing up to four-folds increase in lung tumor retention, and doubling mortality rates. Blood storage time was the critical determinant of these deleterious effects, regardless of whether the transfused blood was allogeneic or autogenic. Surprisingly, aged erythrocytes (9 days and older), rather than leukocytes or soluble factors, mediated the effects, which occurred in both operated and non-operated animals. The effects of erythrocytes transfusion in the MADB106 model emerged immediately, and dissipated within 24 hours.

Conclusions—In rats, transfusion of fresh blood is less harmful than transfusion of stored blood in the context of progressing malignancies. Further studies should address mediating mechanisms through which erythrocytes' storage-duration can impact the rate of complications while treating malignant diseases and potentially other pathologies.
Introduction

Throughout the history of medicine, the beneficial outcomes of allogeneic blood transfusion have been coupled with adverse reactions, including host responses to incompatible red cell determinants, infections, and transfusion-associated immune modulations. Notably, patients receiving blood transfusion prior to organ transplantations have long been reported to exhibit improved graft and patient survival. With additional research it became evident that allogeneic leukocytes present in the transfused blood often suppress host cellular immune responses, particularly those mediated by T lymphocytes and natural killer (NK) cells. This suppression is believed to underlie reduced host-anti graft responses, and increased patients susceptibility to infections.

Cancer progression was also suggested to be affected by blood transfusion. Animal studies conducted by Blajchman et al. elegantly indicated a cancer-promoting effect of allogeneic blood transfusion, employing various animal models. These studies also suggested the involvement of recipients’ cellular immune mechanisms in mediating these effects. In humans, the majority of retrospective and some prospective studies have indicated poorer prognosis in cancer patients receiving allogeneic blood transfusion during the perioperative period. These findings were reported in patients harboring gastric, colorectal, lung, head, neck, prostate, and breast cancers. This suggests the generalizability of the findings, irrespective of cancer type or specific hospital routine.

However, given ethical considerations in human studies, it cannot be determined whether blood transfusion per se, during the perioperative period, is an independent risk factor for postoperative cancer recurrence. It is feasible that the circumstances necessitating blood transfusion, rather than the transfusion itself, underlie such outcomes. Randomized clinical trials that were performed compared different regimens of transfusion, including leukocytes depletion vs. non depletion. Thus, these trials could not have assessed the role of blood constituents that cannot be removed from the transfusion, specifically red blood cells (RBC), and could not have indicated the independent impact of the transfusion as a whole.

An additional unresolved issue is the potential role of donors’ leukocytes in mediating cancer progression in transfused patients. Whereas allogeneic leukocytes were implicated in mediating various adverse effects of blood transfusion, there is no conclusive evidence that they can worsen cancer progression. A large randomized clinical trial comparing leukocytes-containing to leukocytes-depleted regimens concluded that allogeneic leukocytes have no effect on colorectal cancer recurrence, as both conditions showed similar decline in survival rates compared to non-transfused patients. Other studies have similarly reported no beneficial effects of leukodepletion in cancer patients.

In the current study we aimed at addressing some of the unavoidable shortcomings of human studies. Specifically, we (i) tested whether blood transfusion is an independent risk factor for cancer progression, (ii) studied the impact of indispensable blood constituents, including RBC, and (iii) studied the impact of blood storage duration in allogenic and autogenic transfusion. To this end, we employed two non-immunogenic tumor lines syngeneic to the F344 rat used herein: the MADB106 mammary adenocarcinoma, and the CRNK-16 leukemia. We tested cancer progression by monitoring rats’ ability to clear circulating cancer cells and by assessing survival rates. The impact of blood transfusion in the context of surgery was also studied.
Materials and Methods

Animals and counterbalancing

F344 rats (females or males) were purchased from Harlan Laboratories (Jerusalem, Israel), and housed four per cage under 12-h light/12-h dark cycle with free access to food and water. Animals were acclimatized to the vivarium for at least 4 weeks before experimentation and were 12 to 20 weeks old at that time. In any given experiment, all animals were of the same age and sex. To minimize experimental stress, all rats were handled daily for 3 days before each study. The order of blood transfusion, and tumor injection was counterbalanced across groups in each experiment. Control animals were transfused with saline or syngeneic fresh blood. Donors were 6 months old male Wistar (allogenic) or F344 rats (syngeneic/autogenic) housed under the same conditions. The characteristics of the donors (age, sex, etc.) were counterbalanced across recipient groups. All studies were approved by The Institutional Animal Care and Use Committee of Tel Aviv University (Tel-Aviv, Israel), and performed in accordance with relevant guidelines and regulations.

Blood collection, preparation of blood constituencies for transfusion, and storage

Donor rats were overdosed with halothane, and approximately 18 ml of blood from each Wister rat (allogenic blood) or 11 ml of blood from each inbred F344 rat (syngeneic/autogenic blood) was drawn from the heart into syringes containing citrate-phosphate dextrose solution with adenine (Sigma, Rehovot, Israel) (1:7 citrate: blood v/v). For preparation of packed cells, blood was centrifuged for 20 min at 850 g, the supernatant was disposed, and the remaining packed cells were stored at 4°C. For leukoreduction, the serum fraction and the leukocyte buffy coat were removed following centrifugation by manual pipette aspiration. Remaining leukocytes were then counted in each sample before transfusion. To separate leukocytes from RBC before storage, we first used a modified ficoll-paqueplusplus (Amersham biosciences, Uppsala, Sweden) separation procedure. Specifically, 10 ml blood was diluted with equal volume of saline, and placed on 12 ml of ficoll-paque solution in 50 ml tubes. Samples were centrifuged for 10 min at 750 g, and the layers above the RBC, which contained most of the leukocytes, were collected. Leukocytes remaining on top of the RBC fraction were removed manually by a pipette, which also removed approximately 20% of the RBC volume that was discarded. Leukocytes and RBC were then washed 3 times in 40 ml phosphate buffered saline and stored as packed cells. All procedures were conducted under sterile conditions. Unlike standard ficoll-paque separation procedures, this procedure enables collecting most of the leukocytes, including granulocytes. Fluorescence-activated cell sorting analysis and hemocytometric assessment indicated that the harvested leukocytes contained 70%-90% of original leukocytes number, including all leukocytes subpopulations. The RBC fraction contained less 10% of the original leukocyte number, including all leukocytes subpopulations. For leukodepletion – to thoroughly remove leukocytes from the blood we used high efficiency leukocyte removal filters (Pall Purecell NR) (Pall, Portsmouth, United Kingdom) that are routinely used clinically and known to reduce leukocytes by a factor greater than 10^5. Using these filters, we routinely inspected for remaining leukocyte and were able to verify that at least 99.99% of leukocytes were removed.

Transfusion of blood constituencies, and tumor inoculation

Immediately before transfusion, blood constituents (i.e., packed cells, only RBC, only leukocytes, only supernatant from stored packed cells) were filtered through a 40 μm membrane, to remove or break up potential aggregates. F344 rats were anesthetized with halothane (vaporizer, 2.5%) and a 24GA i.v. cannula was inserted into the tail vein. A standard 3 ml volume containing different quantities and fractions of saline-diluted blood constituents was slowly transfused during a 10 min period. Three ml of saline containing the same quantities of citrate-phosphate dextrose solution with adenine as in the transfused constituent was used.
for control transfusion. Unless otherwise indicated, the transfusion content originated from 3 ml of the donor’s blood. Tumor cells were inoculated at the end of this 10 min period through the i.v. cannula (except in the first experiment, in which tumor was given at different time points).

Tumor cell lines and tumor models

MADB106 tumor line—MADB106 is a selected variant cell line obtained from a pulmonary metastasis of a mammary adenocarcinoma chemically induced in the inbred F344 rat [15]. The MADB106 tumor metastasizes only to the lungs following its intravenous inoculation, and is known to be sensitive to NK cells activity in vivo [15–19]. Following intravenous inoculation of MADB106 tumor cells, metastases are formed only in the lungs, and lung tumor retention of MADB106 cells is an early indicator of this outcome [15,17,18]. Cells were maintained at 5% CO₂, 37°C, 100% humidity, in monolayer cultures in complete medium (RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 50 μg/ml gentamicin, 2 mM L-glutamine, 0.1 mM non-essential amino acids and 1 mM sodium pyruvate). Cells were separated from the flask using 0.25% trypsin.

Radiolabeling of MADB106 cells and assessment of lung tumor retention—DNA radiolabeling of tumor cells was accomplished by adding 0.4 μCi/ml of 125Iododeoxyuridine to the growing cell culture 1 day before harvesting the cells. 4×10⁵/kg labeled MADB106 tumor cells in 0.5 ml of phosphate buffered saline were injected into the tail vein under halothane anesthesia. Tumor cells were always administered immediately following transfusion (excluding the first study), and twenty-one hours after tumor inoculation, rats were euthanized with halothane, and their lungs were removed and placed in a γ-counter for assessment of radioactive content. The percentage of tumor retention was calculated as the ratio between radioactivity measured in the lungs and total radioactivity in the injected tumor cell suspension. Our previous studies have indicated that the levels of lung radioactivity reflect the numbers of viable tumor cells in the lungs [17].

CRNK-16 tumor cells—The CRNK-16 cell-line is derived from a naturally occurring leukemia that is highly malignant and is a major cause of natural death in aged F344 rats [20]. CRNK-16 cell line was maintained in complete medium at 100% humidity, 5% CO₂ at 37°C.

Inoculation of CRNK-16 cells and assessment of survival—Under halothane anesthesia, 60 CRNK-16 cells were injected into a rat tail vein in 0.5 ml of phosphate buffered saline immediately following blood transfusion. Beginning a week following tumor inoculation, rats were daily inspected for morbidity. Specifically we euthanized rats that became indifferent or unresponsive to environmental stimuli, that showed motor difficulties, or that lost more than 10% of their body weight. We inspected euthanized rats for the development of solid tumors in internal organs (specifically spleen, liver, kidney, and all organs in the chest cavity) or spinal cord, and identified a malignant development in all morbid rats. Based on our experience with this tumor model [19], these malignancies characterize a progressive stage of this cancer, causing death in the approximating 24–48 hours. Thus, these animals are included in the mortality report, and were considered to have died on the next day. No morbidity was detected past day 82, and rats were inspected until day 112. None of the survivors showed any signs of illness throughout the study.

Surgical procedure (laparotomy)

Anesthesia was induced with halothane and maintained at a concentration of 2–3%. After hair trimming and scrubbing with alcohol, a four-centimeter midline abdominal incision was performed, and the small and large intestines were externalized and covered with phosphate buffered saline-soaked gauze. Before closing, the intestines were repositioned, and the skin...
and muscle were closed in one layer with 4 or 5 sutures. The procedure requires 20 min to conduct.

Statistical analysis

For the MADB106 experiments, analysis of variance was conducted, using % lung tumor retention as the dependent index. Provided significant group-differences existed, two-side protected least significant difference contrasts for pair-wise group comparisons were used. Data is always presented as mean ± SEM. For the CRNK-16 survival study, the Kaplan-Meier model was used for survival analysis, followed by the pair-wise two tailed Tarone-Ware test of group comparisons. For all experiments, a p value of less than 0.05 was considered significant and all p values were two-tailed. StatView 5.0 (Cary, NC) statistical package was used for statistical analysis.

Results

Immediate and short-lasting effect of blood transfusion

To test whether and for how long blood transfusion can affect the clearance of MADB106 tumor from the lungs, allogenic blood stored as packed cells for 12 days (or saline for control) was transfused at 24, 4, 1 or 0 hr before MADB106 tumor cells inoculation, or 1 hr after tumor inoculation. Blood transfusion significantly increased % lung tumor retention up to 5 fold compared to saline transfusion (p < 0.05) when given at all time points (e.g., from 0.055 ± 0.012 to 0.259 ± 0.044, at the 0 hr time point) (p < 0.05), except when given 24 hr before the tumor, in which blood transfusion did not cause any elevation in lung tumor retention (and no significant effect) (fig. 1). This indicates an immediate and short-lasting deleterious effect of blood transfusion on this index of cancer progression.

Effect of storage interval and histocompatibility

To test whether different storage intervals and histocompatibility influence the effect of blood transfusion, allogenic or autogenic blood were stored as packed cells for 0, 3, 9, 12, or 14 days before transfusion. Blood of either allogeneic or syngeneic origin stored for 9 days or longer significantly increased % lung tumor retention (p < 0.05) in a storage-time dependent manner compared to saline transfusion (e.g., saline = 0.150 ± 0.015, 0 days storage = 0.120 ± 0.013, and 14 days storage = 0.501 ± 0.082 in the allogeneic transfusion) (p < 0.05) (fig. 2). Fresh blood, allogeneic or syngeneic, had no deleterious effect.

Effects of cellular and soluble fractions

To determine whether the effects in the previous two studies are mediated by stored cells of the transfusion, or by soluble factors secreted by them, we tested each component separately. Aliquots of allogenic packed cells stored for 14 days were used to extract the supernatant, the cellular fraction without the supernatant (following three washes), or were reconstituted in saline before transfusion (packed cells). The results indicated that packed cells as well as the washed cellular fraction (washed packed cells) significantly increased % lung tumor retention (p < 0.05) more than 3 fold (e.g., from Saline = 0.068 ± 0.005 to Washed packed cells = 0.226 ± 0.049) (p < 0.05), whereas soluble factors in the supernatant had no deleterious outcome (fig. 3). The study was replicated twice yielding the same results.

RBC but not leukocytes mediate the impact of blood transfusion on MADB106 lung tumor retention

To assess the role of RBC and distinguish it from leukocytes, we used three approaches. In the first, we conducted leukoreduction in some but not in other allogeneic blood samples before a 14-day storage period. Increasing quantities of leuko-reduced RBC and similarly handled non-
leuko-reduced packed cells were transfused and compared to saline and to untouched packed cells. The results clearly indicated that the number of leukocytes was not the factor that predicted the deleterious effects of the transfusion, but rather the volume of transfused RBC, irrespective of whether leukoreduction was conducted. Most illustrative is the comparison between the transfusion of the highest RBC volume in the leukoreduction groups, and the transfusion of the lowest RBC volume in the non-leukoreduction groups (indicated in fig. 4 by the horizontal lines). These two groups had the same number of leukocytes (fig. 4A), but the first showed a 5-fold increase in lung tumor retention (from Saline = 0.113 ± 0.025 to 2250 ± 0.584 ± 0.103) (p < 0.05), whereas the second showed a non significant 1.3-fold effect (to 0.153 ± 0.048) (fig. 4B). These findings suggest that RBC volume, rather than leukocyte number, determine the deleterious effects we observed thus far.

To further negate the role of leukocytes we employed a second approach in which we directly assessed the impact of isolated allogeneic leukocytes transfusion. Leukocytes were separated before a 14-day storage period, and compared to similarly stored packed cells and to saline. Fluorescent activated cell sorter analysis indicated that the transfused leukocytes contained all their subpopulations. The results indicated that stored leukocyte transfusion did not cause any increase in % lung tumor retention (fig. 5A), while packed cells caused an approximately 3-fold increase (from Saline = 0.205 ± 0.023) to Packed cells = 0.588 ± 0.060) (p < 0.05). These findings negate a role for stored leukocytes (or factors they secrete) in mediating the metastasis-promoting effects evident herein.

Last, to validate the critical role we ascribed to RBC, we took a third approach that relies on administering RBC thoroughly depleted of leukocytes. To this end we used the standard clinical procedure of filter-based pre-storage leukodepletion that eliminated more than 99.99% of leukocytes (see “Materials and Methods”), and compared it to packed cells and to saline transfusion. The results indicated that both the leukodepleted RBC (mean = 1.036 ± 0.152) and the packed cells (mean = 1.173 ± 0.124) caused approximately 3-fold increase in % lung tumor retention compared to saline (mean = 0.398 ± 0.121) (p < 0.05) (fig. 5B).

The effects of RBC transfusion in the context of surgery and blood loss

To start testing the relevance of our finding to the clinical setting, we also studied the impact of stored RBC transfusion in the context of surgery and blood loss. Animals either underwent laparotomy or were only anesthetized, and were further subdivided and transfused during surgery/anesthesia with pre-storage (14-day) leukodepleted packed cells (only RBC) or with saline. Just before transfusion, 1 ml of blood was withdrawn from all rats by cardiac puncture. The results indicated that RBC transfusion increased MADB106 lung tumor retention in both operated and non operated rats (fig. 6). In fact, beyond the already elevated risk caused by surgery itself (from 0.356 ± 0.041 to 0.702 ± 0.125), RBC transfusion caused greater increase in lung tumor retention in operated rats (mean = 2.169 ± 0.316) compared to non-operated rats (mean = 0.912 ± 0.158), as indicated by a significant interaction between blood transfusion and surgery (two-way analysis of variance indicated significant main effects of surgery (F(1,40) = 13.3, p < 0.05) of transfusion (F(1,40) = 21.2, p < 0.05), and a significant interaction (F(1,40) = 4.3, p < 0.05).

Effect on survival in the CRNK-16 tumor model

To investigate the effects of blood transfusion in the CRNK-16 tumor model, allogeneic blood was either separated to the RBC and the leukocytes fractions, or was not separated, and stored for 14 days as packed cells. Rats were transfused with stored leukocytes, stored packed RBC, or stored packed cells, and were compared to rats transfused with fresh syngeneic (autogenic) packed cells and to rats not transfused. Each transfusion originated from 6 ml of blood and was given in the standard 3 ml volume. The results indicated that both RBC and packed cells...
transfusion significantly reduced survival rates (fig. 7) \((p < 0.05)\). On the other hand, the leukocyte transfusion group was very similar to the two control groups (fresh autologous transfusion, and no transfusion), falling between them. These findings using CRNK-16 leukemia model support our previous results in the MADB106 model indicating that the stored RBC, but not stored leukocytes (or substances they secrete), are responsible for the deleterious effects of stored blood transfusion.

**Discussion**

The leading hypothesis regarding the deleterious impact of blood transfusion on cancer progression considers allogeneic leukocytes to be the major mediating agent \(^7,^{11}\). Accordingly, leukodepletion is a common prophylactic procedure \(^7\). Here, for the first time, we show that in two animal models donors’ RBC, rather than leukocytes or soluble factors, can be a critical constituent underlying the cancer promoting effects of blood transfusion. We further show that these effects of RBC critically depend on the duration of blood storage, irrespective of donors’ histocompatibility. Specifically, in our models both autologous and allogeneic blood transfusions increased cancer progression when stored for more than nine days, whereas fresh blood, allogeneic or syngeneic, had no deleterious effects. Storage is known to result in the deterioration of RBC \(^{21}\), which we believe substantially contributed to their evident cancer promoting effects.

The medical context of blood transfusion in cancer patients and the involvement of immunity in the potential deleterious effects of the transfusion should be considered when evaluating the clinical relevance of this study. In cancer patients, blood is often transfused around the time of excising the primary tumor. As was recently reviewed by others and us, this perioperative period is characterized by numerous processes that induce an abrupt elevation in the risk for the outbreak of preexisting micrometastases and the seeding of new metastases \(^{22}\). Most relevant to our framework, malignant tissue is notoriously non-cohesive, and surgical procedure often disrupts the neoplasm or its vascularization, leading to a release of tumor cells into the circulation \(^{23,24}\). As a matter of fact, recent studies indicate that most cancer patients harbor single cancer cells after the removal of the primary tumor \(^{25}\). In the current study, the two tumor models used simulate the presence of circulating cancer cells, as well as a sudden elevation in the risk of cancer progression in conjunction with blood transfusion. A second aspect of tumor-host interaction that is relevant to the context of blood transfusion is the role of cellular immunity, particularly T cells and NK cells, in controlling minimal residual disease \(^{25}\). Many primary tumors evolve to become non-immunogenic, or develop other escape mechanisms to evade adaptive immunity. Cytotoxic T cells and NK cells have been reported to interact with the malignant tissue along this process, to restrict metastases, and to play a role in eradicating residual cancer after the primary tumor has been removed \(^{26–29}\). In the current study, the two tumor models used are non-immunogenic, and sensitive to the in vivo activity of NK cells \(^{15,17–19}\), an important aspect of innate immunity. Further supporting the potential role of NK cells in the current study are the findings that the cancer-promoting effects commence immediately upon RBC transfusion and without prior exposure to the tumor. Thus, if immunity is involved, it is innate immunocytes, which can react immediately upon first encounter with tumor cells \(^{16}\). Overall, the tumor models used herein and the study of RBC transfusion to operated rats reflect important aspects characterizing the clinical setting of blood transfusion in cancer patients, and the potential relevance of innate cellular immunity (e.g., NK cells) in eliminating residual cancer cells.

It is questionable whether tumor models, even the most advanced, can indeed simulate the prolonged and complex processes of human cancer evolvement and interactions with the patient immune system and other physiological constituents. Therefore, animal studies employing tumor models can only suggest mechanisms and potential clinical outcomes, and

"Anesthesiology. Author manuscript; available in PMC 2009 December 1."
these suggestions should eventually be tested in humans. The tumor models that we have used, each has its advantages and shortcomings. The MADB106 experimental metastasis model does not entail a primary tumor, hence its shortcoming as a model. However MADB106 tumor cells are well characterized with respect to susceptibility to specific immunocytes (e.g., NK cells) and the time course of this susceptibility (up to 24 hr postinoculation). Thus it is used herein to suggest immune mediation of the effects, and their exact time dependency. This model is also used to simulate the final stages of tumor extravagation and survival in a target organ. In fact, the lung tumor retention of MADB106 cells is an early indicator of the number metastases to be formed in the lungs weeks later, as indicated with respect to the effects of NK depletion, ethanol intoxication, adrenergic and prostaglandin challenges, and the impact of surgery. The second tumor model used herein, the CRNK-16 leukemia, may be considered as having greater clinical relevance with respect to blood-born tumor progression. The CRNK-16 line originated from a naturally occurring leukemia that is highly malignant and is the major cause of natural death in senescent F344 rats. As is common in many human malignancies, this tumor expresses low levels of major histocompatibility complex-I, and do not evoke effective immunological memory. Thus, the herein orthotopic implantation of CRNK-16 tumor cells enable the in vivo study of cancer progression in a biologically relevant setting, which may also bear clinical significance.

Stored or deteriorating RBC transfusion may impact blood-born cancer progression via numerous mechanisms, immunological or non-immunological. The involvement of host immune mechanisms was suggested by several studies, but the specific mediating potential of donor RBC was not addressed. It could be hypothesized that deteriorating RBC, which were reported to alter membrane determinants, may preoccupy host innate immune effector cells, leaving tumor cells unattended. Provided that as few as 0.1% of the transfused RBC would become targets to host immunocytes, these RBC will outnumber residual/circulating tumor cells by several order of magnitude, and will probably outnumber relevant host immunocytes. This would dramatically reduce the chances that a host immunocyte would interact with a residual tumor cell and eliminate it. A nonexclusive hypothesis may address the role of host cytokines and hormones. Deteriorating RBC are approached and eliminated by host splenic and hepatic leukocytes that also control the host milieu of various soluble factors. Specifically, following blood transfusion there is a reduction in splenocyte secretion of IL-2, and elevation in monocytes-derived systemic levels of prostaglandin E2. Both perturbations are known to suppress cellular immunity, NK cells in particular. Last, it is noteworthy that both hypotheses presented in this paragraph are equally relevant to autologous and allogenic deteriorating RBC, which indeed did not differ in their deleterious effects in the current study.

Several limitations of our study should be considered. As already noted, animal models of cancer cannot be expected to simulate the much longer and perhaps more complicated course of cancer evolvement and progression in humans. The tumor models used herein simulate only some aspects of human host-cancer interactions, involving some, but not other relevant immune and non-immune mechanisms. Additionally, our outcomes are focused on relatively short-term consequences of blood transfusion, with the exception of survival rates in animals challenged with the CRNK-16 leukemia. With respect to the transfused blood, although both human and rat RBC were reported to deteriorate during storage in citrate-phosphate dextrose solution with adenine, rat RBC exerted quicker alterations in several parameters. Thus, the exact time course of storage-dependent deleterious effects should be studied in human blood. Last, it was beyond the scope of the current study to reveal molecular mediating mechanisms of the deteriorating RBC in rats or in humans. Therefore, more studies are needed in order to better understand the phenomenon and devise optimal prophylactic approaches.
The clinical implications of our results could, nevertheless, be significant. Although RBC transfusion cannot be altogether avoided, the use of freshly drawn blood should be tested in cancer patients. Further research in animal models and in cancer patients is required to determine mediating mechanisms, specifically the role of host innate immunity, the cytokine response, and non-immunological mechanisms. Other animal studies implicated allogenic leukocytes in promoting cancer progression. However, unlike in the current study, in these earlier studies blood was transfused several days before or several days after exposure to the tumor, and thus these earlier studies are likely to reflect different mediators. Taken together, different mechanisms could be involved and may impact different aspects of host-tumor interactions in different time frames. Irrespective of mediating mechanism, we suggest that donors’ leukocytes might not be responsible for every adverse outcome of blood transfusion, and that the universal procedure of leukodepletion may not be sufficient to overcome the deleterious effects of blood transfusion in cancer patients. Long-stored RBC should be studied as a potential risk factor in cancer and non-cancer patients. A combined approach employing leukodepletion in freshly drawn blood may prove to be a safer alternative in the context of oncological surgeries, as well as in other surgeries involving high risk of infection and complications, and thus deserves further clinical studies.

Acknowledgments

Financial support: Support was provided solely by NIH/NCI CA125456 grant (S. B-E) and by a grant from the Israel Science Foundation (S. B-E), Jerusalem, Israel.

References


Figure 1. Blood transfusion increases MADB106 lung tumor retention when transfused in close time proximity to tumor inoculation

% of lung tumor retention (mean± SEM) in rats transfused at several time point before MADB106 tumor inoculation (-24, -4, or -1 hours), simultaneously with tumors (0), or 1 hr after tumor inoculation (+1) compared to corresponding saline transfusion. n = 44, 3–6 per group. * indicates significant pair-wise difference from the same time point saline control group (protected least significant difference, p < 0.05).
Figure 2. Both allogeneic and autogenic blood transfusions increased lung tumor retention in a storage time-dependent manner.
% of lung tumor retention (mean± SEM) in rats transfused with saline, allogeneic blood (Allotransfusion), or autogenic blood (autotransfusion) that were stored for various durations as packed cells. * indicates significant pair-wise difference from the saline control group (protected least significant difference, p < 0.05). n = 104, saline & 9 days 12–14 per group, other groups 4–8.
Figure 3. Cells but not supernatant of stored blood increase MADB106 lung tumor retention
% of lung tumor retention (mean± SEM) in rats transfused with 14-days stored packed cells, post-storage washed packed cells, post-storage supernatant from packed cells (supernatant), or saline. n = 22, 4–8 per group. * indicates significant pair-wise difference from the saline control group (protected least significant difference, p < 0.05).
Figure 4. Red blood cell (RBC) cell volume, but not leukocyte number, determines the metastasis promoting effects of the transfusion

(A) Numbers (mean± SEM) of leukocytes per a transfusion containing 0, 0.75, 1.5, or 2.25 ml of RBC (diluted in saline to a standard 3 ml volume). Transfusions were conducted following 14-day storage of leukoreduced or non-leukoreduced packed cells. Notice that the two groups contrasted by the horizontal bar have similar number of leukocytes but different RBC volume.

(B) % of MADB106 lung tumor retention (mean± SEM) in the different groups. Notice that the same two groups contrasted are significantly different from each other in tumor retention (indicated by **, protected least significant difference, p < 0.05), although having the same number of leukocytes (A). * indicates significant pair-wise difference from the respective saline control group (protected least significant difference, p < 0.05). n = 67, 6–10 per group.
Figure 5. Leukocytes do not increase MADB106 lung tumor retention, whereas leukodepleted red blood cells (leukodepleted packed cells) do increase it.

% of lung tumor retention (mean± SEM) in rats transfused with: (A) Packed cells, but not leukocytes significantly increased tumor retention. n = 24, 6–9 per group. (B) Both packed cells and leukodepleted-packed cells (only red blood cells) significantly increased tumor retention. n = 21, 6–8 per group. * indicates significant pair-wise difference from the respective saline control group (protected least significant difference, p < 0.05).
Figure 6. Stored leukodepleted packed cells (red blood cells - RBC) increase MADB106 lung tumor retention in operated rats more than in non-operated rats

% of lung tumor retention (mean± SEM) in rats undergoing or not undergoing surgery, and transfused with stored leukodepleted packed cells (RBC) or with saline. Surgery significantly increased % of lung tumor retention, and blood transfusion further increased it, causing a significantly greater impact in operated than in non-operated animals (p < 0.05). n = 44, 7–9 no surgery groups, 14–16 surgery groups.
Figure 7. Transfusion of stored blood, specifically stored red blood cells (RBC), reduced survival rates in CRNK-16-derived leukemia

Transfusion of stored packed cells and transfusion of stored leukodepleted packed cells (only RBC) significantly reduced survival rates (Tarone-Ware test, p < 0.05), while transfusion of leukocytes or fresh autogenic packed cells had no effect. * indicates significant pairwise difference from the control group. n = 163, 28–39 per group.