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Enhanced Growth of Artificial Tumor Metastases Following Blood Transfusion: The Effect of Erythrocytes, Leukocytes and Plasma Transfusion

S.K. SINGH, R.L. MARQUET, D.L. WESTBROEK and J. JEEKEL

Department of Surgery and Laboratory for Experimental Surgery, Erasmus University, PO Box 1738, 3000*DR*, *Rotterdam, The Netherlands*

Abstract—*Clinical and experimental investigations have shown that allogeneic blood transfusions may modulate the growth of tumors. Dependent on the tumor model used in animal studies, the effects observed ranged from inhibition to stimulation of tumor growth. We have demonstrated previously that allogeneic blood transfusions gave rise to enhanced growth of a transplantable sarcoma (LS* 175*) in BN rats. In the experiments reported here the effect of transfusion of different allogeneic blood constituents on the growth of artificial LS* 175 *lung metastases was investigated. Erythrocytes and leukocytes were found to promote tumor growth to a similar degree as whole blood transfusions, plasma transfusions had no effect.*

INTRODUCTION

CLINICAL and experimental results have shown a prolongation of renal allograft survival, following pre-operative blood transfusions [1–4]. Investigations into which blood components could be responsible for the immuno-suppression after blood transfusions have produced conflicting results. Lymphocytes, erythrocytes and platelets have been shown to be effective by some [5–9], while others were unable to obtain similar results [10]. Recent retrospective studies have indicated a link between blood transfusion and shorter disease free interval of cancer patients [11, 12]. Employing different host–tumor models, experimental studies have shown reduced, stimulated or no effect on tumor growth following blood transfusions [13, 14]. Previously, we have demonstrated enhanced growth of artificial tumor metastases following allogeneic whole blood transfusion, in a rat tumor model [15, 16].

In the experiments reported here, we have investigated the competence of different components of blood in modulating metastatic growth. Effect of erythrocytes, leukocytes and plasma transfusion on tumor growth is compared with the effect observed after transfusion of whole blood.

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MATERIALS AND METHODS

Animals

Male rats of the inbred BN and WAG strains were used. The animals were bred under specific pathogen free conditions and were 16–20 weeks old.

Tumor

Tumor LS 175 is a spontaneous, non-immunogenic sarcoma in BN rats. The tumor is maintained as a stationary culture in Dulbecca's minimum essential medium, supplemented with 10% fetal calf serum (FCS). To obtain cells for the *in vivo* experiments free floating LS 175 cell clumps were harvested from the tissue culture flasks and, after washing, were resuspended in Hank's balanced salt solution (HBSS). Single cells were prepared by rinsing the suspension through a pipette. Viability was assessed by trypan blue exclusion and was between 90 and 95%.

Lung colonies assay

Tumor cells (2.5×10^5), suspended in a volume of 1 ml, were injected i.v. into experimental and control rats. The number of colonies developing in lungs was counted after 24 days. The lungs were excised, rinsed in tap water and subsequently fixed in Bouin's solution. Tumor nodules on the lung surface, visible to the naked eye, were counted.

Transfusions

Whole blood. BN rats received a single i.v. injection of 1 ml heparinized whole blood.

Erythrocytes. Heparinized WAG rat whole blood was centrifuged for 10 min at 200 ***g***, the buffy coat removed and the sediments pooled and passed in HBSS over a cotton wool column, as described by Diepenhorst *et al.* [17]. BN rats received an i.v. injection of an equivalent number of erythrocytes present in 1 ml whole blood (5×10^9), suspended in a volume of 1 ml of HBSS. This suspension was contaminated with < 1% leukocytes found in normal blood.

Leukocytes. Whole blood was diluted 1 : 2 with HBSS. Leukocytes were obtained by centrifugation of diluted blood into lymphocyte separation medium (LSM, Bionetics). Residual erythrocytes were eliminated by three successive incubations of the leukocyte enriched suspension, with hemolytic buffer (Tris-buffered ammonium chloride 0.17 M) and subsequently washed in HBSS. This procedure resulted in marked reduction in the number of erythrocytes (< 0.1%). The viability of the remaining leukocytes was determined by trypan blue exclusion test, and appeared to be > 95%). About 1×10^7/ml leukocytes, equivalent to the number of cells in 1 ml of whole blood, were suspended in HBSS and 1 ml of the suspension was administered i.v. to BN rats.

Plasma. Heparinized WAG rat whole blood was centrifuged for 10 min at 1500 ***g*** and the resulting supernatant collected. The supernatant was subsequently passed through a filter of 0.45 μm gauze (Schleicher & Schull, FPO30/2). Half a milliliter of the filtered plasma was administered i.v. to BN rats.

Experimental design

Blood constituents responsible for the stimulation of tumor metastatic growth were investigated. BN rats were inoculated with 2.5×10^5 LS 175 tumor cells, on day 0. On day +7 groups of rats received i.v. 1 ml of either whole WAG rat blood or WAG erythrocyte suspension, leukocyte suspension or 0.5 ml of plasma, respectively. Control animals received no transfusion. The animals were sacrificed on day +24 and lung colonies assay was performed. The assay was statistically analysed using Student's *t* test, with the limit of significance $P < 0.05$.

RESULTS

The resulting lung colonies on lung surface on day +24 following experimental procedures on day +7, are illustrated in Table 1. The mean number of lung colonies of group of animals administered whole blood is 68 ± 7. Animals which did not receive any treatment, the control group, showed 17 ± 6 lung colonies. The number of colonies in the control group is significantly less than the whole blood transfused animals. Groups of animals administered erythrocyte and leukocyte suspension showed 57 ± 2 and 68 ± 8 mean number of colonies, respectively. These numbers are also statistically significantly higher than those found in control animals. The numbers found in the experimental groups were similar to one another.

The group of animals receiving plasma transfusion showed 53 ± 11 lung colonies (Table 2). The control group of plasma transfused animals, not receiving any transfusion, showed 54 ± 12 lung colonies, i.e. there was no difference between these groups.

DISCUSSION

Different blood components have been shown to be successful in the attainment of effective immunosuppression in organ transplant models [5–8]. Jenkins and Woodriff [10] have been able to achieve prolongation of cardiac allografts in rats by lymphocyte transfusates, but were unable to obtain the same effect by an erythrocyte transfusion. Jeekel *et al.* [5] found purified donor erythrocyte transfusion capable of inducing indefinite survival of rat kidney allografts. Francis and Shenton [13] have demonstrated stimulated tumor growth and depressed lymphocyte reactivity in laboratory animals, after whole blood transfusion. We have shown similar reduced immunocompetence and metastatic growth [16, 18]. The blood donor WAG rat differs for class I and class II antigens, with respect to the BN tumor bearing rats. Erythrocytes in the rat express class I histocompatibility antigens of the A locus at the cell surface but do not express any detectable class II antigenic determinants (B and D locus products). Leukocytes express both class I and II antigens. Erythrocyte and leukocyte suspensions were equally efficient in stimulating tumor growth as was the whole blood transfusion. This suggests that class I antigens alone or combined with class II antigens are capable of producing the blood transfusion effect observed here. The small degree of contamination of the erythrocyte suspension with leukocytes might explain the observed effect of erythrocyte transfusion. However, Wood *et al.* [19] have also shown suppression of the immune apparatus following highly purified erythrocyte suspension. Kapnick and Monaco [8] have shown that platelets transfusions, which also lack class II antigens, are also capable of inducing unresponsiveness to skin allografts in mice. Oikawa *et al.* [20], in a study of a host–tumor model where allogeneic whole

Table 1

EFFECT OF WHOLE BLOOD, LEUKOCYTES AND ERYTHROCYTES ON THE GROWTH OF ESTABLISHED TUMOR METASTASES

Transfusion on day +7	Number of animals	Mean number of lung colonies
Washed leukocytes	9	68 ± 8
Washed erythrocytes	10	57 ± 2
Whole blood	10	68 ± 7
No transfusion	10	17 ± 6

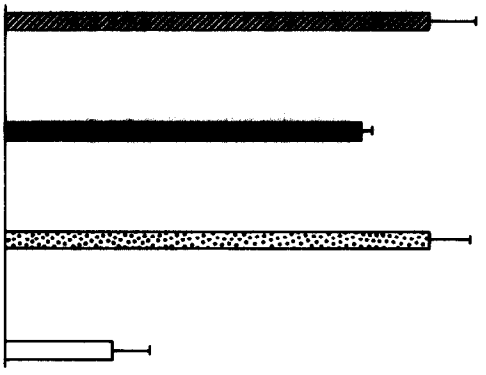
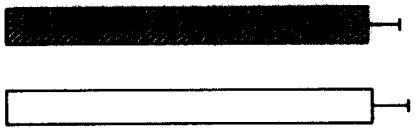


Table 2

EFFECT OF PLASMA TRANSFUSION ON GROWTH OF ESTABLISHED TUMOR METASTASES

Transfusion on day +7	Number of animals	Mean number of lung colonies
Plasma	10	53 ± 11
No transfusion	9	54 ± 12



blood transfusion resulted in reduced tumor growth, observed a similar effect following administration of red blood cells, leukocytes and platelets transfusions. This observation and our results here suggest that different blood components are capable of producing similar results as are obtained following whole blood i.v. transfusion, in a particular host–tumor model. In analyses of the retrospective data of surgically treated cancer patients, Blumberg *et al.* [12] found a relationship between greater amount of blood and blood components transfused and higher incidence of recurrence and death. An explanation could be the presence of a substance in plasma capable of reducing immune function in the transfused patients. Margolese and Wainberg [21] have demonstrated a poor response of breast cancer patient lymphocytes to T cell growth factor, in the presence of viruses. Furthermore, growth of transplanted tumors in mice is shown to be greatly enhanced following plasma transfusion [22]. Francis and Shenton [13] have also reported an increase in plasma suppressive activity after allogeneic transfusion and not following syngeneic or saline infusion in rats. However, our results do not support the hypothesis of the presence of a substance capable of modulating tumor growth.

To summarize, erythrocyte and leukocyte transfusions are capable of producing enhanced growth of tumor metastases, the effect being similar to whole blood transfusion. Plasma transfusion has no effect on tumor growth. Class II antigens do not appear to be a crucial factor in precipitating the blood transfusion effect, since the same effect was observed after transfusion of erythrocytes bearing only Class I antigens.

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