

Migration of Transfused Granulocytes in Leukopenic Dogs

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Although granulocyte transfusion therapy has been shown to be effective in infected granulocytopenic animals and humans, the relative effectiveness of granulocytes (PMN) harvested by continuous flow centrifugation (CFC) or by continuous flow filtration leukapheresis (FL) remains uncertain. Studies in vitro of morphology and granulocyte functions have suggested cells collected by FL may be damaged. To compare the function in vivo of granulocytes collected by different methods, dogs were made granulocytopenic with cyclophosphamide (CYT) and then transfused with granulocytes collected by CFC or FL. The local neutrophil mobilization (LNM)

through a standard skin abrasion into a chamber containing a strong chemoattractant, autologous serum, was measured. Greater LNM was found after transfusions of CFC PMN than after transfusions of the same number of FL PMN ($p < 0.0003$). This difference persisted even when the dose of FL PMNs was four times greater than that of CFC PMN and when the FL donor was pretreated with steroids ($p < 0.001$). These results suggest that during filtration leukapheresis, granulocytes are functionally altered and that their function in vivo may be compromised.

GRANULOCYTE TRANSFUSION THERAPY has been shown to be effective in infected granulocytopenic animals and humans.¹⁻⁷ Granulocytes may be collected for transfusion by continuous flow centrifugation (CFC)^{1,2} or by continuous flow filtration leukapheresis (FL).^{3,8,9} The technique of filtration leukapheresis yields larger numbers of granulocytes (PMN) from normal donors in less time and for less cost than continuous flow centrifugation. However, recent studies suggest FL PMN may be altered by the collection technique. Cells collected by FL appear morphologically abnormal^{9,10} and do not circulate normally after transfusion.¹¹⁻¹³ In vitro assays of FL neutrophil functions have yielded conflicting results. Some studies have found alterations in chemotactic, phagocytic, and bactericidal capacities of FL neutrophils;^{9,14} other studies have found no functional deficiencies.^{15,16} These studies have not approached the question of the function in vivo of FL neutrophils. Whether deficits in cell function which have been documented by assays in vitro persist after transfusion and hinder the ultimate migratory potential of transfused cells remains untested. Senn and others have developed a simple skin chamber technique for quantification of local neutrophil mobilization. Migration of cells into this skin chamber appears to correlate with the early phase of granulocyte defense against bacterial infection.^{17,18} This technique has been applied in an experimental animal system in order to compare the relative capability for extravascular migration of cells collected by FL and CFC.

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MATERIALS AND METHODS

Beagles weighing 7–12 kg, 6–12 mo old, were used for measurement of local neutrophil mobilization. These animals were chosen because of our previous experience with the effects of cyclophosphamide on these dogs and because their size allows for easy handling. Ten normal dogs comprising group A received no therapy. All other animals were made granulocytopenic by the intravenous infusion of CYT (40 mg/kg). Beginning 24 hr after the CYT infusion and continuing until the animals recovered their granulocyte counts, all dogs were supported with ampicillin 500 mg intramuscularly (i.m.) b.i.d., gentamicin 25 mg i.m. b.i.d., and daily Lactated Ringer's solution, 750 ml, by subcutaneous clysis. All dogs receiving CYT had local neutrophil mobilization (LNM) measured on day 5 after chemotherapy, a time at which they were severely granulocytopenic. Five dogs (group B) received no granulocyte transfusions, while dogs in groups C, D, E, and F did.

Male English-American foxhounds were used as granulocyte donors because their mild temperament and large size (20–25 kg) relative to the smaller beagle recipients simplified the collection of the doses of granulocytes used in this study. Granulocytes were obtained by either continuous flow centrifugation or by filtration leukapheresis as previously described.^{6,19} For both methods, donors had indwelling arteriovenous shunts inserted 24 hr prior to the leukapheresis procedure. To collect PMN by CFC, animals were leukapheresed for 3 hr using a cell separator (NCI-IBM cell separator, Endicott, N.Y.) obtaining 150 ml of leukocyte-rich ACD anticoagulated suspension. When FL was the procedure used, the peristaltic pumps incorporated in the cell separator were used to pump blood at 80 ml/min over two nylon-wool filters (Leukopak filters, Fenwal, Morton Grove, Ill.). After 2 hr, elution was accomplished using 20% ACD NIH formula A plasma in saline with gentle tapping. The number of PMNs contained in each collection was measured and the volume adjusted to contain the dose of PMN required for that experiment. The suspensions were kept at room temperature and transfused within 2 hr of collection. One group of donors was treated with 4 mg dexamethasone orally 12 hr before leukapheresis. These methods paralleled those used for human granulocyte collections at the National Cancer Institute.

LNM was measured as described by Senn.^{17,18} An area on the dog's flank, prepared by shaving and disinfected with betadine, was washed with ethanol. The area was allowed to dry and stretched taut. A homogeneous abrasion of 1 sq cm size was made by sliding a grinding cylinder rotating at high speed (Handee grinder, Chicago Wheel Co., Chicago, Ill.) over the skin. The area was then covered with a sterile soft silicon rubber chamber, taped in place, and 2 ml of autologous serum were injected into the chamber through the self-sealing wall. Although we tried other fluids, autologous serum was used as the chamber fluid in these experiments because we found, in agreement with others, that autologous serum provided a stronger, more reproducible attraction than any other fluid tested. After 24 hr, the chamber fluid was removed and the leukocytes and PMN (usually > 95% of the total leukocytes) in the fluid counted. The final LNM was defined as the number of granulocytes per milliliter that collected in the chamber fluid over a 1 sq cm abrasion during the 24-hr test period. If the dog was to be given a granulocyte transfusion, it was infused just after the abrasion was completed at the start of the test period.

Statistical comparisons between means were by two-tailed *t* tests on the logarithms of the data in order to stabilize variances.²⁰ The linear regression was performed by the usual methods.²¹

RESULTS

Granulocytopenia was repeatedly produced by the fifth day after the CYT, as shown in Fig. 1. Circulating PMN levels on day 5 were 0–415 PMN/cu mm (mean = 215). In ten normal dogs (group A), the LNM obtained was 49.7×10^6 PMN/sq cm/24 hr which was comparable to the values reported in humans of 24.5×10^6 PMN/sq cm/24 hr by Perillie and Finch,²² and of 73.1×10^6 PMN/sq cm/24 hr found by Senn and Jungi.¹⁸

As shown in Table 1, granulocytopenic dogs not transfused (group B) had a very low LNM. Transfusion of 4×10^8 PMN/kg collected by FL did not

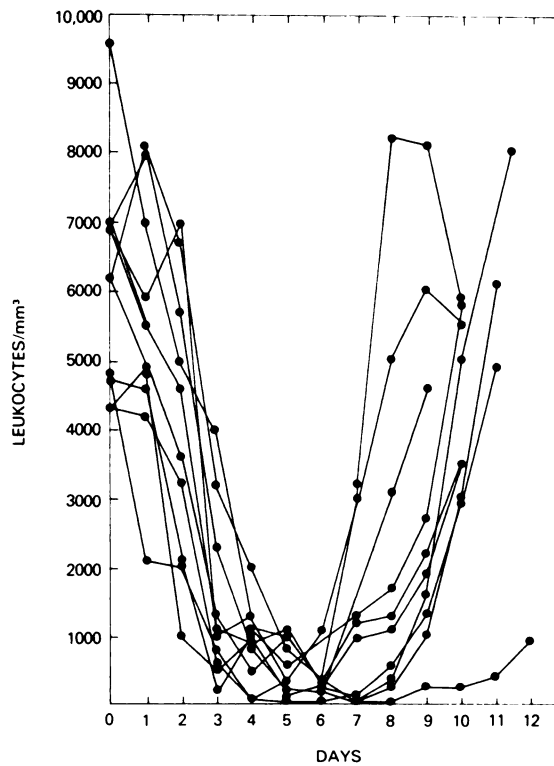


Fig. 1. Peripheral leukocyte counts in ten beagles after the infusion of 40 mg/kg cyclophosphamide.

result in a larger LNM (group C), but transfusion of 4×10^8 PMN/kg collected by CFC (group F) caused a significant increase in LNM ($p < 0.0003$). The LNM afforded by 4×10^8 CFC PMN/kg was also greater than that produced by the infusion of 16×10^8 FL PMN/kg with or without prior treatment of the donor with steroids ($p < 0.001$). Extrapolation of these data by linear regression predicted that 32×10^8 FL PMN/kg would be required to produce the same LNM provided by a transfusion of 4×10^8 CFC PMN/kg (see Fig. 2).

DISCUSSION

Filtration leukapheresis is clearly an efficient and inexpensive method for granulocyte collection. Recent studies have suggested that the collection tech-

Table 1. Local Neutrophil Mobilization (LNM) Into Autologous Serum in 32 Dogs Grouped According to Treatment and Granulocyte Transfusion

Group	No. of Dogs	Treatment	PMN Transfusion/kg	LNM* (PMN $\times 10^6$ /sq cm/24 hr)
A	10	None	None	49.70 ± 5.35
B	5	CYT	None	0.17 ± 0.030
C	5	CYT	4×10^8 FL	0.16 ± 0.088
D	3	CYT	16×10^8 FL	1.20 ± 0.520
E	4	CYT	16×10^8 FL + Steroids	1.62 ± 0.230
F	5	CYT	4×10^8 CFC	5.94 ± 1.14

*Mean \pm SEM.

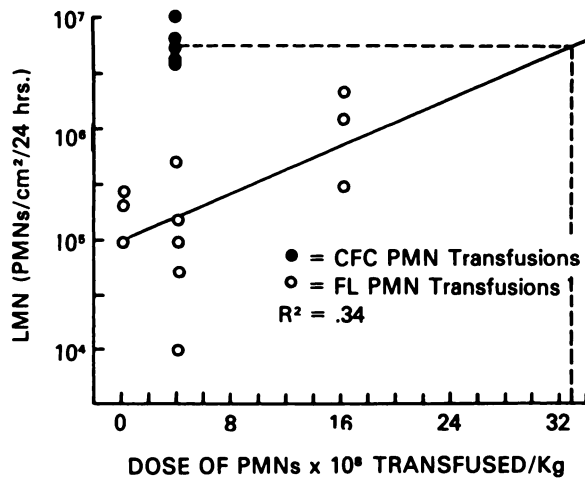


Fig. 2. Regression of \log_{10} LNM as a function of the dose of FL PMN transfused. Regression represented by the solid line is extended until it intersects the LNM achieved by the transfusion of 4×10^8 CFC PMN/kg. This would suggest that 32×10^8 FL PMN/kg would be required to achieve this LNM if the regression curve remained linear at this dose.

nique may damage the cells. Herzig,⁹ Aisner,¹⁰ and Han²³ have described morphological alterations in FL PMN consisting of vacuolation and cytoplasmic disruption. Fehr,²⁴ Debelak,¹² Herzig,¹³ and Meuret¹¹ have all suggested that when compared to CFC cells, FL cells give lower transfusion increments with possible increased margination and sequestration in the spleen and lungs. Some studies of phagocytosis, bactericidal killing, and chemotaxis have shown cells collected by FL to be defective when compared to normal,^{9,14} while other studies have demonstrated no such deficiencies.^{15,16} It is also of note that transfusion reactions appear more common following transfusion with FL PMN than following CFC PMN infusions.¹

This study demonstrates a workable model for examining one in vivo function of granulocytes by measuring the ability of the cells to migrate from the microcirculation to a strong chemoattractant following transfusion into a granulocytopenic recipient. Using this model, it appears that cells collected by CFC are more capable of migration than cells collected by FL. Harris¹⁵ and Wright¹⁴ have suggested that the least damage to human PMNs collected by FL occurs when collection times are less than 2 hr, and when the donors are pretreated with steroids. In this study these suggestions have been employed, yet a functional deficiency appears to persist.

The precise mechanism by which cells are damaged during FL remains unsettled. Kloch and Bainton have demonstrated decreased in vitro bactericidal power of granulocytes collected by FL, which they relate to active degranulation of the granulocyte during its adherence to nylon wool.²⁵ Wright et al.²⁶ have shown that it is the small peroxidase-negative specific granule that seems to be preferentially lost during the granulocyte's adherence to nylon wool. It remains unclear whether the adherence and degranulation of granulocytes during FL damages cells directly or sensitizes them to further damage by other means. What does seem clear is that the damage demonstrated in vitro is not repaired after transfusion, and that these cells neither circulate normally intravascularly nor migrate normally extravascularly.

This study has not addressed the overall efficacy of FL cells for the treatment

of infections, since it has already been shown that FL cells are effective in both experimental and clinical situations. The major intent of these experiments has been to investigate more completely the relative functional capacities of CFC and FL PMN in order to understand better how to employ and modify each of the collection techniques. It appears that a significantly larger dose of FL PMN may be required to achieve a therapeutic response equal to that of a smaller dose of CFC PMN. Studies aimed at improving the quality of cells obtained using the FL technique would seem warranted. A model similar to that described here may be useful in evaluating these refinements.

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