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The relationship between alloimmunization and posttransfusion granulocyte survival: experience in a chronic granulomatous disease cohort

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Abstract

BACKGROUND—The efficacy of granulocyte transfusions in patients with HLA alloimmunization is uncertain. A flow cytometric assay using dihydrorhodamine 123 (DHR), a marker for cellular NADPH oxidase activity, was used to monitor the differential survival of transfused oxidase-positive granulocytes in alloimmunized patients with chronic granulomatous disease (CGD).

STUDY DESIGN AND METHODS—Ten patients with CGD and serious infections were treated with daily granulocyte transfusions derived from steroid and granulocyte–colony-stimulating factor–stimulated donors. The proportion of neutrophils with intact oxidase activity was quantitated by DHR fluorescence on samples drawn before and 1 hour after transfusion. The incidence of acute transfusion reactions was correlated with the results of DHR fluorescence and biweekly HLA serologic screening assays.

RESULTS—Eight of 10 patients experienced acute adverse reactions in association with granulocyte transfusions. Four had only chills and/or fever, and four experienced respiratory compromise; all eight exhibited HLA alloimmunization. Mean ($\pm$ SD) oxidase-positive cell recovery was $19.7 \pm 17.4\%$ ($n = 15$ transfusions) versus $0.95 \pm 1.59\%$ ($n = 16$) in the absence and presence of HLA allosensitization, respectively ($p < 0.01$). Greater than 1% in vivo recovery of DHR-enhancing donor granulocytes was strongly correlated with lack of HLA alloimmunization.

CONCLUSION—The ability to detect DHR-positive donor granulocytes by flow cytometry is strongly correlated with absence of HLA alloimmunization and lack of acute reactions to granulocyte transfusions in patients with CGD. If HLA antibodies are present and the survival of donor granulocytes is low by DHR analysis, transfusions should be discontinued, avoiding a therapy associated with high risk and unclear benefit.

Chronic granulomatous disease (CGD) is a heterogeneous group of inherited disorders characterized by recurrent, often life-threatening, pyogenic infections.^{1–3} In CGD, abnormalities of the NADPH oxidase system result in failure of neutrophils to produce reactive oxygen intermediates.^{1–4} The lack of these oxygen species impairs the ability of neutrophils to destroy invading microorganisms and leads to granuloma formation.

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Interferon- γ (IFN- γ) is used prophylactically to prevent infections through mechanisms that are not well defined.⁵ Treatment of established infections in CGD is based on administration of antibacterial or antifungal therapy and surgical drainage when appropriate. Granulocyte transfusions have been given to augment the body's defense mechanisms when infections are severe or antimicrobial therapy is thought to be inadequate.^{2,6-8} Other modes of treatment under investigation include hematopoietic stem cell transplantation and gene therapy to incorporate normal genes for the oxidase system into hematopoietic stem cells.^{9,10}

Although granulocyte transfusions are used empirically to treat patients with infections resulting from CGD, the efficacy of this treatment in patients who become alloimmunized is uncertain. Alloimmunization to both HLA and neutrophil-specific epitopes occurs in up to 78% of CGD patients who have received granulocyte transfusions,^{7,11} and these antibodies have been shown to destroy transfused granulocytes.¹²⁻¹⁴ Thus, a single course of granulocyte transfusions can adversely affect the ability to tolerate or obtain benefit from subsequent courses. Even in those who are not alloimmunized, it is difficult to evaluate the respective contributions of granulocytes and antimicrobials to improvements in the patient's condition.

Alloimmunization is not the only risk associated with granulocyte transfusions. Acute systemic and pulmonary reactions, including fever, rigors, and respiratory distress, are well documented and are more common in the presence of alloimmunization.^{7,15-17} The risk of transmission of infectious agents, although very small, is present with any blood component.

Despite the ability to prospectively identify alloimmunized patients, it has been difficult to predict which patients will gain clinical benefit from a course of granulocyte transfusions. Under certain conditions, it may be desirable to administer granulocytes to alloimmunized recipients if the potential benefits of therapy are assessed as outweighing the risks. A method that quickly and accurately identifies patients in whom acute severe reactions are likely to occur and clinical benefit would be unlikely would greatly enhance our ability to determine the best course of action.

A flow cytometric assay using dihydrorhodamine 123 (DHR) can detect the presence of intact NADPH oxidase activity in neutrophils and quantitate the ability of such cells to undergo a normal respiratory burst.¹⁸⁻²¹ Since the respiratory burst is impaired in granulocytes from CGD patients, this assay can be used to quantitate and monitor the survival of transfused oxidase-positive granulocytes in these patients. We used the DHR assay as a supplement to HLA serologic screening in the identification of patients at high risk of acute transfusion reactions and at low likelihood of clinical benefit from such transfusions. Ten patients with CGD and life-threatening infections were serially evaluated with HLA serum screens and quantitative DHR assays during the course of daily granulocyte transfusion therapy.

MATERIALS AND METHODS

Patient cohort

Ten patients with CGD and ongoing infections were selected for clinical reasons to receive a course of daily granulocyte transfusion therapy. The clinical decision to pursue granulocyte transfusion support was made by the primary care physician, without taking into consideration HLA serostatus or history of prior granulocyte transfusions. All patients received conventional antimicrobial therapy for bacterial or fungal infections. The clinical status of the patients was monitored using history, vital signs, and pulse oximetry before, during, and immediately and 1 hour after the transfusions. The transfusions were continued

until the patient's condition improved to the point of hospital discharge, a severe transfusion reaction occurred, or the patient died.

Granulocyte collections

Granulocyte products were collected by continuous-flow centrifugation (Model CS3000 Plus cell separator, Baxter Healthcare, Round Lake, IL) using trisodium citrate as the anticoagulant (Tricitransol 46.7%, Cytosol Laboratories, Braintree, MA) and 6% hetastarch as the red blood cell (RBC) sedimenting agent (Hespan, B. Braun Medical, Irvine, CA).^{22,23} The instrument interface offset was set at 33. Volunteer donors received either 8 mg of dexamethasone orally, 5 µg/kg granulocyte colony-stimulating-factor (G-CSF, filgrastim, Amgen, Thousand Oaks, CA) subcutaneously, or both dexamethasone and G-CSF, 12 to 18 hours before apheresis.^{24,25} Approximately 6.5 L of whole blood was processed per donation. The concentrates were stored for less than 10 hours at 22°C, without agitation. Since patients with CGD are immunocompetent, the granulocyte components were not irradiated before transfusion.

HLA antibody testing

Serum screening for HLA antibodies was performed using a standard lymphocytotoxicity assay before initiation of granulocyte transfusions and at biweekly intervals during granulocyte transfusion therapy.²⁶

Neutrophil antibody testing

Neutrophil antibody testing was performed using a monoclonal antibody immobilization of granulocyte antigen assay.¹¹

¹¹¹Indium-oxine white blood cell trafficking studies

Two patients underwent white blood cell (WBC) trafficking studies using ¹¹¹indium-oxine-labeled allogeneic cells during the course of the granulocyte transfusions.^{12,13,27} An aliquot of 5×10^9 donor granulocytes was labeled with 10 µCi ¹¹¹indium-oxine per kg recipient weight, with the total radionuclide dose not to exceed 500 µCi. After several washes to remove unbound radionuclide, the labeled cells were injected intravenously into the patient. Nuclear imaging scans were performed at 24 and 48 hours after injection.

Absolute neutrophil counts

Absolute neutrophil counts (ANCs) were obtained from peripheral blood samples before and 1 hour after granulocyte transfusions. Corrected count increments (CCIs) for ANC were calculated from the formula

$$CCI = \frac{(\text{posttransfusion ANC} - \text{pretransfusion ANC}) \times BSA}{\text{No. granulocytes transfused}}$$

where CCI is corrected count increment, ANC is absolute neutrophil count (cells/µL), BSA is body surface area (m²), and the number of granulocytes transfused is stated as cells $\times 10^{10}$.

Flow cytometry

Blood samples were obtained immediately before and 1 hour after granulocyte transfusions. Flow cytometric studies using DHR were performed as previously described.^{18–21} In normal phagocytes, stimulation of membrane-bound NADPH oxidase by exposure to phorbol myristate acetate results in the conversion of oxygen to superoxide (O₂[−]) and hydrogen peroxide (H₂O₂). DHR is a fluorescent dye that localizes in the mitochondria and, after

oxidation by O_2^- and H_2O_2 to rhodamine 123, emits a bright fluorescent signal upon excitation by blue light (488 nm). WBCs were isolated by centrifugation from 300 μ L of whole blood after dilution and RBC lysis. The cells were washed and 5 μ L of a stock catalase solution (1400 U/ μ L, Sigma, St Louis, MO) was added to 400 μ L of resuspended cells to yield a final concentration of 1000 U/mL. A 29 mmol/L stock solution of DHR 123 (Molecular Probes, Eugene, OR) was prepared and 1.8 μ L was added to each reaction tube for a final DHR concentration of 1.0×10^5 nmol/L.²¹ After incubation at 37°C for 5 minutes, 100 μ L of a stock phorbol myristate acetate solution (3.2×10^3 nmol/L, Sigma) was added. After a final 15-minute incubation at 37°C, the samples were analyzed by flow cytometry.

Samples were analyzed on a flow cytometer (FACSort, BDIS, San Jose, CA) using its accompanying software (Lysis II, BDIS). Granulocytes were identified by forward and side scatter and confirmed using CD45 and CD14 (BDIS); fluorescent FL2 (580 nm) signal was also analyzed.²¹ Samples were run in the setup mode until a granulocyte acquisition gate was established, at which point 10,000 events in the granulocyte gate were collected. Autofluorescence in the absence of DHR is negligible in this system.²¹

Initial DHR fluorescence intensity was expressed as a percentage of total events. The absolute number of DHR-enhancing cells was obtained by multiplying the ANC by the percentage of cells with DHR enhancement. Because CGD granulocytes do not undergo fluorescent enhancement with DHR, the absolute number of DHR-enhancing cells was taken to be the number of circulating donor granulocytes.

CCIs for DHR-enhancing cells were calculated from the formula:

$$CCI = \frac{(\text{posttransfusion AbsDHR} - \text{prettransfusion AbsDHR}) \times BSA}{\text{No. granulocytes transfused}}$$

where CCI is corrected count increment, AbsDHR is absolute number of DHR-enhancing cells per μ L, BSA is body surface area (m^2), and number of granulocytes transfused is stated as cells $\times 10^{10}$.

Statistical analysis

Comparisons of tests on paired samples from the same donor were performed with a paired, two-tailed t test. Comparisons between groups were conducted with a two-tailed, nonpaired t test. Differences in occurrence of acute transfusion reactions between groups were compared with Fisher's exact test. Regression coefficients were computed using a spreadsheet application (Excel, Microsoft Corp., Redmond, WA).

RESULTS

Patient cohort

The 10 patients with CGD ranged from 4 to 23 years old; nine were male (Table 1). Three had fungal pneumonias, three had staphylococcal liver abscesses, two had bacterial pneumonias, and two had nocardial infections. Five of the 10 had received prior granulocyte transfusions (Table 2).

Effect of donor stimulation

The cellular content of the apheresis components ranged from 1.3×10^{10} to 11.3×10^{10} granulocytes (mean $\pm$ SD, $5.25 \times 10^{10} \pm 2.79 \times 10^{10}$). As expected, components produced

after dexamethasone administration contained the least number of cells; mobilization with G-CSF plus dexamethasone resulted in a nearly fourfold increase in granulocyte content compared with products produced using dexamethasone alone (Table 3).^{24,25}

Incidence of HLA and neutrophil alloimmunization

Three patients (Patients 4, 5, and 9) were already HLA alloimmunized at the start of the granulocyte transfusions and showed a multispecific, broadly reactive pattern on lymphocytotoxicity screening (Table 2). Five additional patients (Patients 1, 6, 7, 8, and 10) developed strong and broadly reactive HLA antibody screens within 2 days to 2 weeks of starting transfusions, indicative of an anamnestic response. All five had received granulocyte transfusions or nonleukoreduced RBC transfusions in the past. One of the two remaining patients (Patient 2) exhibited weak and inconsistent reactions involving less than 15% of the panel cells, and the other patient (Patient 3) consistently had a negative antibody screen.

Neutrophil-specific antibodies reactive against CD16, NA1, and NB1 were found in three patients (Patients 7, 9, and 10). Since these patients also exhibited HLA allosensitization, the role of neutrophil-specific antibodies in causing adverse effects could not be determined.

Quantitation of circulating donor granulocytes by DHR staining

DHR assays were performed before and after 31 of 263 granulocyte transfusions in the 10 patients studied (Table 3). Mean ($\pm$ SD) oxidase-positive cell recovery was $19.7 \pm 17.4\%$ ($n = 15$) in the absence of HLA allosensitization versus $0.95 \pm 1.59\%$ ($n = 16$; $p < 0.01$) in the presence of HLA antibodies. There were no acute reactions associated with any granulocyte transfusion in which the post-transfusion detection of oxidase-positive donor cells was greater than 1% ($n = 17$ transfusion events in seven patients). HLA alloantibodies were not detected before any of these 17 transfusions.

The converse was also true. All three patients (Patients 4, 5, and 9) who consistently exhibited less than 1% posttransfusion recovery of DHR-positive cells had strongly positive preexisting HLA antibody screens. In two of these three patients, severe acute pulmonary transfusion reactions occurred after 2 to 33 transfusions, necessitating discontinuation of transfusions ($p < 0.05$ for comparison of acute transfusion reactions in patients with CCI_{DHR} of greater than 1% vs. reactions in those with CCI_{DHR} of less than 1%).

Five patients developed a multispecific pattern of HLA alloimmunization during the course of granulocyte transfusions, probably due to an anamnestic response. In one of these patients (Patient 6), the posttransfusion detection of DHR-positive cells fell from 11% after the first week of granulocyte transfusions to 0.6% at the time of HLA antibody detection (Table 3). This decrease in percentage of circulating oxidase-positive donor cells was associated with the development of a severe pulmonary transfusion reaction requiring cessation of transfusion therapy. In Patient 1, the posttransfusion recovery of DHR-positive cells fell from 5.4% after the first week of transfusions to 0.01% 2 weeks later at the time of HLA antibody detection. This was accompanied by mild fever and chills during 19 of the remaining 50 transfusions. In the other three patients, the DHR assay could not be repeated after HLA alloantibodies were detected, since no further granulocytes were given.

Pre- and posttransfusion ANC were available for 23 of the 31 transfusions (Table 3). There was no correlation between the number of granulocytes administered and the change in ANC. In fact, the ANC did not always increase after transfusion. In 18 of 23 granulocyte transfusions, an increment in ANC was seen; in the remaining five, a decrement was noted. There was also no correlation between the change in ANC and the change in absolute

number of DHR-positive cells after transfusion. This analysis held true for transfusions both in the presence (n = 13) and in the absence (n = 10) of documented HLA antibodies.

¹¹¹Indium-oxine trafficking studies

Allogeneic ¹¹¹indium-oxine WBC trafficking studies were performed in two patients. In one patient (Patient 2), lack of trafficking to the lungs in the presence of a high in vivo corrected DHR-enhancing cell increment suggested that the pulmonary infection had resolved and that only scar tissue remained. Patient 5 demonstrated a lack of trafficking to known sites of cutaneous nocardial disease. In combination with a very low in vivo DHR-enhancing cell recovery, this indicated that HLA alloantibodies were destroying the ability of transfused granulocytes to traffic to sites of infection. Studies in both patients were used to guide cessation of therapy.

Incidence of acute adverse reactions to transfusion

Acute adverse transfusion reactions occurred in 8 of 10 patients receiving daily granulocyte transfusions (Table 2). In four patients (Patients 1, 4, 7, and 10), these reactions were mild to moderate in severity, consisting of fever, chills, or allergic symptoms. However, the other four patients (Patients 5, 6, 8, and 9) experienced acute respiratory insufficiency, with fever, rigors, dyspnea, cyanosis, and significant declines in oxygen saturation. All eight patients had broadly reactive HLA antibody screens and five had virtually absent in vivo recovery of DHR-positive cells (less than 1%) before these reactions (in three, the DHR assay was not repeated after HLA antibodies appeared). However, it was not possible to predict, either by the number of panel reactive cells in the lymphocytotoxicity screen or by the DHR assay, which patients would have the most severe reactions. No adverse reactions were seen in the two patients who did not develop HLA allosensitization.

Clinical outcome

Nine of 10 patients eventually cleared their infections and were discharged from the hospital, after 2 to 41 transfusions (Table 2). One patient with disseminated *Aspergillus nidulans* expired. In four patients, granulocyte transfusions were continued until it was felt that the infection had resolved. In five, severe adverse transfusion reactions were the proximate cause of stopping the transfusions.

DISCUSSION

The role of granulocyte transfusions in the treatment of CGD remains controversial. The availability of newer treatment options, including IFN- γ and potent antibacterial and antifungal agents, has markedly improved the quality of life and decreased the number of hospital days for these patients. However, recurrent serious infections still occur. In view of the paucity of controlled data showing a survival benefit for recipients of granulocyte transfusions, the risks of granulocyte transfusions must be examined carefully. The majority of CGD patients survive infectious episodes even in the absence of this therapy, yet mortality in CGD is still about 2% to 3% per year at our institution.

A very high proportion of CGD patients receiving granulocytes will develop HLA alloimmunization: 78% (14 of 18) in a recent retrospective study¹¹ and 80% (8 of 10) in the current study. Allosensitization substantially increases the risk of serious reactions to subsequent transfusions and significantly impairs trafficking to sites of infection. In a meta-analysis of the efficacy of granulocyte transfusions, survival benefit could only be demonstrated in recipients receiving WBC crossmatch-compatible cells.²⁸

Not all HLA-allosensitized patients experience serious transfusion reactions. In this study, two of eight alloimmunized individuals experienced only mild and occasional chills or low-grade fevers. In patients who tolerate such transfusions well despite alloimmunization, it would be of great help to have a simple, rapid diagnostic test to predict the clinical efficacy of the product and the likelihood that a more serious reaction will occur.

We employed a flow cytometric technique to assess the survival of donor granulocytes in patients with CGD. The technique is based on the ability of normal granulocytes to oxidize the nonfluorescent DHR to the fluorescent rhodamine 123 through the respiratory burst. Since patients with CGD have granulocytes that lack NADPH oxidase and cannot mount a respiratory burst, all fluorescing granulocytes are of donor origin. Our results demonstrate that the DHR assay, used in synergy with the HLA antibody screen, was highly informative in assessing the potential efficacy of granulocyte transfusions. The assay demonstrated that 1) a very high recovery of circulating donor cells, up to 50% to 60% of the total circulating neutrophil pool, could be detected in patients without alloimmunization; 2) the percentage of donor cells present after transfusion dropped dramatically concomitant with an anamnestic HLA antibody response; and 3) the percentage of donor cells in the recipient's circulation was extremely low (less than 1% of circulating neutrophils) in all patients with existing HLA alloantibodies. Unfortunately, the DHR assay could not be used to predict which alloimmunized patient would develop the most severe or life-threatening reactions to transfusion.

Differing donor preparative regimens led to large variations in the dose and possibly the quality and immunoreactivity of the granulocyte components used in this study. In neutropenic patients, the increase in granulocyte count after transfusion is directly correlated with the number of cells transfused, and the increase in count serves as a surrogate marker for the efficacy of the transfusion.^{29,30} In the CGD patients in this study, however, there was no correlation between the number of granulocytes transfused and either the posttransfusion increase in granulocyte count or the increase in oxidase-positive cells. Furthermore, patients with significant increments in ANC after transfusion did not necessarily have increments in numbers of oxidase-positive cells. It is possible that in patients with normal numbers of autologous granulocytes, transfusion of allogeneic cells causes a variable degree of mobilization or demargination of native cells into the circulation, obscuring the increase due to the transfused cells alone. It is also possible that HLA antibodies prevented a posttransfusion increment in cells in some patients. However, a wide range in the recovery of donor cells in the DHR assay was seen even in patients without HLA alloimmunization. This could be due to a number of factors, including variations in the quality of the granulocyte product, clinical events in the recipient, and technical imprecision in the performance of the assay. Although no significant differences were seen in the increase in oxidase-positive cells in nonimmunized recipients after transfusion of components mobilized with dexamethasone alone versus G-CSF-containing regimens, the sole oxidase-positive cell recovery of less than 1% in a nonimmunized recipient (0.85% recovery of oxidase-positive cells) occurred after transfusion of 2.22×10^{10} granulocytes mobilized by dexamethasone alone. Thus the differing preparative regimens likely contributed to the wide standard deviation (SD) in the DHR assay data, but did not alter the overall study conclusions.

¹¹¹Indium-labeled WBC trafficking studies may be helpful in identifying sites of infection.^{12,13,27} Lack of specific trafficking in the two radiolabeling studies performed in our CGD cohort was highly informative, not for identifying the site of infection, but for documenting resolution of infection in one patient with a high in vivo corrected DHR increment and a persistently abnormal chest X-ray and for documenting inability to get to sites of known abscess formation in a second patient with a very low in vivo DHR recovery.

The results of trafficking studies in both patients were used as indications to terminate transfusion therapy.

Due to the high incidence of alloimmunization in patients in this study, it was not possible to assess the effect of granulocyte transfusions on clinical outcome of infection. However, 9 of 10 patients successfully cleared their infections and were discharged from hospital, despite the fact that 8 of 10 were alloimmunized and had very poor in vivo recovery of transfused cells. It thus appears that a high proportion of CGD patients may be capable of clearing serious infections with the help of antimicrobial therapy and surgical excision alone. A randomized trial of granulocyte transfusions in patients with CGD is needed to determine the impact of this therapy on the clinical course of infection and on survival.

One weakness of the study was the small number of subjects and the relatively small number of DHR assays performed given the fairly wide SD of the data. A larger confirmatory trial in patients with CGD might be helpful before using these data to alter practice.

We conclude that the DHR assay provides a valuable means to determine whether granulocyte transfusions might be of clinical benefit to patients with CGD, by measuring the in vivo recovery of transfused cells. The absence of circulating cells after transfusion is widely accepted as a surrogate for the absence of potential clinical efficacy. If HLA antibodies are present and the recovery of donor cells is low by DHR analysis, the risks of transfusion are likely to outweigh potential benefits, and transfusions should be discontinued. A high in vivo recovery of circulating DHR-positive cells is strongly correlated with lack of alloimmunization and absence of acute reactions, suggesting that continued transfusions might offer clinical benefit.

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ABBREVIATIONS

ANC(s)	absolute neutrophil count(s)
CGD	chronic granulomatous disease
DHR	dihydrorhodamine 123

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TABLE 1

Characteristics of CGD patients

Patient	Sex	Age (years)	Infection type
1	Male	7	Disseminated <i>Aspergillus nidulans</i>
2	Male	4	<i>Burkholderia cepacia</i> pneumonia
3	Male	21	<i>Staphylococcus aureus</i> liver abscesses
4	Male	18	<i>S. aureus</i> liver abscesses
5	Female	23	Disseminated <i>Nocardia</i>
6	Male	16	<i>S. aureus</i> liver abscesses
7	Male	7	<i>A. nidulans</i> pneumonia
8	Male	8	<i>Nocardia otitidiscaviarum</i> pneumonia
9	Male	17	Fungal pneumonia, <i>Acremonium</i> species
10	Male	4	<i>Burkholderia gladioli</i> pneumonia

TABLE 2

Alloimmunization and incidence of reactions^{*}

Patient	Previous granulocyte transfusion	HLA antibodies	PMN antibodies	After transfusion, %DHR+ (mean)	Total number of transfusions	Total number of reactions [†]	Type of reaction [†]
1	No	Negative → Positive	Negative	1.6	64	19	Mild fever and chills
2	No	Negative	Negative	30.3	25	0	None
3	Yes	Negative	Negative	3.8	22	0	None
4	Yes	Positive	Negative	.49	41	6	Mild chills
5	Yes	Positive	Negative	.23	33	33	Severe pulmonary reaction [†]
6	No	Negative → positive	Negative	5.8	27	6	Severe pulmonary reaction [†]
7	No	Negative → positive	Positive	14.6	32	10	Moderate fever and rigors
8	No	Negative → positive	Negative	18.7	15	4	Fever, rigors, flank pain, O ₂ desat [†]
9	Yes	Positive	Positive	.09	2	2	Severe pulmonary reaction [†]
10	Yes	Negative → positive	Negative → positive	23	2	2	Moderate fever and chills [†]

PMN = polymorphonuclear leukocytes.

^{*} Represent reactions seen over all granulocyte transfusions, not just those studied with DHR cytometry.

[†] Transfusion therapy discontinued due to severity of reaction.

TABLE 3

In vivo recovery of donor granulocytes

Patient	Number*	Number of PMNs transfused ($\times 10^6$)	Before transfusion		After transfusion		Δ ANC ($\times 10^9/L$)	Δ AbsDHR ($\times 10^9/L$)	ANC CCI†	AbsDHR CCI‡
			ANC ($\times 10^9/L$)	DHR+ (%)	ANC ($\times 10^9/L$)	DHR+ (%)				
1	8									
Mean		3.99	4.79	0	7.38	1.55	2.3	0.14	383	31.4
Range		1.82–6.13	3.15–6.43	0	6.02–10.2	0.01–5.4	0.32 to 3.91	0.001 to 0.44	129 to 746	0.2–113
SD		1.59	1.21	0	1.54	2.12	1.68	0.18	261	46.2
2	6									
Mean		5.49	2.76	0.08	5.29	30.31	2.71	1.71	251	191.4
Range		2.22–9.1	1.35–5.06	0–0.3	2.21–9.23	0.85–61.6	–0.11 to 5.55	0.059 to 5.68	–33.5 to 412	4.38–494
SD		2.6	1.36	0.12	2.8	23.34	2.22	2.31	174	194.8
3	2									
Mean		4.69	9.45	0	7.69	2.28	–1.76	0.175	–596	59.2
SD		0.858	3.00	0.51	2.98	5.38	–0.02	0.145	–37	268
4	3									
Mean		2.62	5.13	0.12	3.94	0.49	–0.76	0.02	–1000	13.7
Range		1.3–4.46	3.97–5.99	0–0.36	3.31–4.57	0.01–0.85	–2.12 to 0.6	0 to 0.039	–2420 to 421	0–27.4
SD		1.64	1.04	0.21	0.89	0.43	1.92	0.03	2009	19.4
5	3									
Mean		5.21	6.36	0.06	21.5	0.23	15.81	0.107	3330	22.5
Range		1.52–7.86	5.69–7.02	None	None	0.07–0.51	None	None	None	None
SD		3.29	0.94	0	ND	0.24	ND	ND	ND	ND
6	3									
Mean		9.04	3.89	1.09	4.48	5.81	0.59	0.21	108	41.8
Range		8.81–9.43	3.18–4.4	0–3.09	3.33–5.1	0.6–10.88	–0.77 to 1.92	0.031 to 0.54	–150 to 353	5.7–106
SD		0.34	0.64	1.74	1.0	5.14	1.35	0.28	252	55.7
7	2									
Mean		11.3	2.25	0	3.69	15.7	1.44	0.579	117	46.9
SD		8.54	3.53	0.02	5.39	13.47	1.86	0.725	200	77.8
8	2									

Patient	Number*	Number of PMNs transfused ($\times 10^6$)	Before transfusion		After transfusion		Δ ANC ($\times 10^9/L$)	Δ AbsDHR ($\times 10^9/L$)	ANC CCI†	AbsDHR CCI‡
			ANC ($\times 10^9/L$)	DHR+ (%)	ANC ($\times 10^9/L$)	DHR+ (%)				
9	1	3.71	ND	5.52	6.15	17.96	ND	ND	ND	ND
		8.16	3.95	0.65	6.44	19.4	2.49	1.223	368	181
10	1	4.52	7.04	0	19.36	0.093	12.32	0.018	3070	4.49
		5.61	5.45	0	14.0	23	8.55	3.22	995	375

AbsDHR = absolute DHR; ND = not detected; PMN = polymorphonuclear leukocytes.

* Number of DHR assays. For patients who had only one or two assays, complete original data are listed.

† $(\text{Posttransfusion ANC} - \text{Pretransfusion ANC}) \times \text{BSA}$

No. granulocytes transfused

‡ $(\text{Posttransfusion AbsDHR} - \text{Pretransfusion Abs DHR}) \times \text{BSA}$

No. granulocytes transfused