Therapeutic Apheresis and Dialysis
9(2):137–141, Blackwell Publishing, Inc.

Granulocyte Transfusions in Children With Chronic Granulomatous Disease and Invasive Aspergillosis

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Abstract: The transfusion of granulocytes to restore host defenses in severely granulocytopenic patients or in patients with defective granulocyte functions has been studied for more than 60 years. However, inadequate dosage of cells and inconsistent efficacy has limited the usage of these transfusions. Recently, the use of mobilizing agents such as granulocyte colony stimulating factors and dexamethasone has renewed interest in these treatment modalities. The present study is conducted to determine an appropriate method of enriched granulocyte collection with Fresenius AS.TEC.204 cell separator (Fresenius, Bad Homburg, Germany) and to evaluate the preliminary clinical results of granulocyte transfusion therapy in patients with chronic granulomatous disease and invasive Aspergillosis in parallel with in vitro granulocyte function. Three patients who have been treated for chronic granulomatous disease and invasive Aspergillosis received a total of 20 granulocyte transfusions. To mobilize granulocytes, healthy donors were given 450 μg of granulocyte colony-stimulating factor (G-CSF) subcutaneously and 8 mg of dexamethasone orally approximately 12 h before collection. Five μg/kg/day of G-CSF was also subcutaneously administered prior to granulocyte transfusions. The first patient received 4; the second, 14 and the third, 2 transfusions. The granulocyte count given to these patients ranged between 0.4 and 3.0×10^9/kg. Most transfusions were well tolerated. The nitroblue tetrazolium (NBT) tests that were done 16–24 h after the transfusion showed 14–46% dye reduction. Two of the three patients survived the infection. Granulocyte transfusions from G-CSF and dexamethasone stimulated donors could be a choice of treatment in chronic granulomatous disease patients, especially with disseminated invasive Aspergillosis. **Key Words:** Chronic granulomatous disease, G-CSF, Granulocyte transfusion, Invasive Aspergillosis.

Granulocyte transfusion therapy has long been considered a logical approach to the treatment of severe bacterial and fungal infections in patients with prolonged granulocytopenia or having intrinsic defects in granulocyte function (1). However, because of the inability to collect sufficient numbers of granulocytes from healthy donors and inconsistent efficacy, the benefit of these transfusions has, for years, been limited. The recent use of recombinant granulocyte colony-stimulating factor (G-CSF) and dexamethasone to mobilize granulocytes in donors before leukapheresis, has created interest in the potential clinical applications of granulocyte transfusion therapy (2–4).

Received June 2004; revised October 2004.
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Chronic granulomatous disease (CGD) is a primary immunodeficiency caused by a defect in the generation of the oxidative burst that normally accompanies phagocytosis of microorganisms (5). Invasive Aspergillosis is a devastating infection that usually occurs in the setting of an impaired immune system (6) and is one of the most common causes of serious infection and death in individuals with chronic granulomatous disease. *Aspergillus* infection usually begins in the lungs with pneumonia, and bone involvement by direct extension or hematogeneous spread may also occur (7). Despite specific antifungal agents and surgical treatment, the mortality rate due to invasive Aspergillosis in CGD is still high. Transfusion of healthy granulocytes is an effective method for treating infections associated with CGD (5,8,9).

Here, we report three CGD patients with invasive pulmonary Aspergillosis, paravertebral abscesses and osteomyelitis. A method of enriched granulocyte collection with Fresenius AS.TEC.204 cell separator

(Fresenius, Bad Homburg, Germany) has been used to evaluate the value of granulocyte transfusion therapy in patients with chronic granulomatous disease and invasive Aspergillosis.

MATERIALS AND METHODS

Patients

Three patients who were being treated for CGD and invasive pulmonary Aspergillosis received a total of 20 granulocyte transfusions.

Patient I

A 3-year-old boy was referred to the Department of Pediatric Allergy–Immunology of Ankara University Medical School, Ankara, Turkey, with symptoms of recurrent skin and soft tissue abscesses. He was diagnosed with CGD. He was put on itracanazol and trimethoprim-sulfamethoxazole prophylaxis but he neither came for follow-up nor received drugs on a regular basis. Fifteen months later he was admitted to the hospital with complaints of persistent fever and draining abscesses at multiple parts of his body. On physical examination, he was found to be tachypneic and had crepitating rales at the base of the lungs together with 5 cm liver and 4 cm spleen enlargement. There were draining abscesses at the back of the neck (15 cm × 15 cm), the left axillary (1 cm × 1 cm) and the sacral (3 cm × 3 cm) region. Radiological examination revealed pneumonic consolidation at the left lung, paravertebral abscess formations at the T3-T5 vertebras together with severe destruction and collapse of the T5 vertebra.

Patient II

A 4-year-old boy who had developed empyema and chest wall abscess formation after an inguinal hernia operation was diagnosed with CGD in our department.

Cervical and inguinal lymphadenopathies (<2 cm in diameter); liver and spleen enlargements (2 and 3 cm, respectively) were detected on his physical examination. He was mildly tachypneic with minimal intercostal retractions. Thoraco-lumber computed tomography (CT) scans revealed multiple granulomatous formations located at the left lung area and a paravertebral abscess at L1. Left pneumectomy was performed by two consecutive surgeries in addition to systemic multiagent antibacterial and antifungal therapies.

Patient III

A 2.5-year-old girl was admitted to our clinic with a draining abscess on the sternum, paraparesis and pneumonia. She has been diagnosed as CGD with a negative nitroblue tetrazolium (NBT) test. A CT scan of the thorax revealed pneumonic consolidation at the left lung, paravertebral abscess formation at C7-T4 and destruction of adjacent bones.

Clinical features, detected microorganisms and sites of applied treatments are given in Table 1 for all three patients.

DONORS

Donors were selected from among volunteering family members and patient relatives. Their ABO

TABLE 1. *Clinical features and treatment of patients*

	Patient I	Patient II	Patient III
Age (years)	3	4	2.5
Age at diagnosis (years)	2.5	4	2.5
Site and type of infection	Left lung Paravertebral abscess at T3-T5 osteomyelitis	Left lung Paravertebral abscess at T10-L1 osteomyelitis	Left lung Paravertebral abscess at C7-T4 osteomyelitis
Type and site of detected microorganisms	*Aspergillus fumigatus*, *Staphyloccocus aureus* and *Escherichia coli* in draining abscess, *Corynebacterium* in blood culture	*Aspergillus fumigatus* in lung tissue	*Aspergillus flavus* and *Staphyloccocus aureus* in draining abscess, *Acinetobacter baumannii* in blood culture
Treatment			
1. Antibacterial treatment	Teicoplanin + Amikacin	Vancomycin + Imipenem	Vancomycin + Meropenem
2. Antifungal treatment	Liposomal Amphotericin B	Liposomal Amphotericin B, Itraconazol	Amphotericin B Itraconazol
3. Surgery	–	Left pneumectomy	Segmentectomy
4. Number of granulocyte transfusions	4	14	2
Outcome	Did not show up for further examination and died 1 year after discharge	Alive and well for 2 years	Died on postoperative day 19, due to ARDS

ARDS, Acute Respiratory Distress Syndrome.

blood groups were compatible with those in respective recipient patients. The donor had adequate peripheral venous access, normal physical examination, normal circulating blood count and had negative serological tests for cytomegalovirus (CMV), human immune deficiency virus (HIV), hepatitis B, hepatitis C, and Veneral Disease Research Laboratory (VDRL) slide test. Each donor received 8 mg of dexamethasone orally and 450 μg of G-CSF (Filgrastim, Roche, Basel, Switzerland) subcutaneously, approximately 12 h before the scheduled granulocyte collection. Informed consent was obtained prior to each mobilization. The donors were also informed that participation would involve the administration of G-CSF subcutaneously (s.c.) in addition to dexamethasone that was given orally.

Blood samples of the donors were obtained by venipuncture before the administration of mobilizing agents and leukapheresis in order to determine white blood cell (WBC) counts.

Granulocyte collections

Granulocyte concentrates were collected by standard centrifugation leukapheresis, using the Fresenius AS.TEC.204 blood cell separator. Approximately 5.2 L of blood was processed within 2–3 h using peripheral venous access for each collection. Trisodium citrate (33.3%, 50 mL) and 500 mL of 6% hydroxy-ethyl-starch (HES; Eczacibaşi, Istanbul, Turkey) were used as anticoagulating fluid and sedimenting agent, respectively, at a ratio of 1/10. A total volume of 200–250 mL is produced in 13 cycles with a speed of 750 mL in each. 400 mL of blood volume is processed in each cycle. This method is obtained from the Price et al. study which has been adapted initially for the Fresenius AS.TEC.204 (1).

Granulocyte transfusions

Leukapheresis products were irradiated with 25 Gy and transfused to patients within a 2–3-h period, soon after collection. G-CSF was also administered to patients (5 μg/kg/day, s.c.) prior to granulocyte transfusions. Patients received acetaminophen (15 mg/kg/dose, per oral) prior to transfusion. Vital signs (temperature, heart rate, respiratory rate, blood pressure) and oxygen saturation were monitored immediately before, during, and 1–2 h after transfusions. Total leukocyte and absolute granulocyte counts of the patients were determined immediately before and 1 h after the completion of the transfusions and on the following morning. Granulocytes were transfused at least 8 h apart from the administration of amphotericin B.

TABLE 2. *White blood cell (WBC) and absolute granulocyte counts (AGC) of the patients before and after granulocyte transfusions*

	WBC ($\times 10^9$/L)	AGC ($\times 10^9$/L)
Before transfusion		
Mean ± SD	15.8 ± 7.0	9.7 ± 6.3
Range	6.4–30.0	4.0–24.6
1 h after transfusion		
Mean ± SD	18.8 ± 12.4	17.5 ± 16.5
Range	8.0–56.7	2.0–50.0
16–24 h after transfusion		
Mean ± SD	19.9 ± 11.3	12.4 ± 8.1
Range	10.7–55.0	6.2–33.3
24–48 h after transfusion		
Mean ± SD	12.6 ± 3.8	8.0 ± 3.4
Range	8.6–20.5	4.6–15.0

RESULTS

Donors were given dexamethasone plus G-CSF before leukapheresis, for each of the 20 granulocyte collections. These agents were well tolerated except for an incidence of mild myalgia. Donor peripheral and WBC counts were $7.2 \pm 1.1 \times 10^9$/L (mean ± SD) before G-CSF/dexamethasone mobilization and $38.1 \pm 6.80 \times 10^9$/L immediately before leukapheresis. The interval between stimulation and leukapheresis was 10–13 h. Twenty leukapheresis procedures were performed with an average yield of $20.7 \pm 10.8 \times 10^9$/L granulocytes (range: 6.4–36.6×10^9/L). An average of $1.7 \pm 0.8 \times 10^9$ granulocyte/kg (range: 0.4–3.0) of patients body weight was given in each granulocyte transfusion. The percentage of lymphocytes in granulocyte concentrates obtained varied between 3.2 and 8.9% with a mean value of 5.2%.

Most of the granulocyte transfusions were well tolerated. However, a transient decrease to the level of 89% in oxygen saturation occurred once during the last transfusion in the second patient.

The mean of the patients' absolute granulocyte counts (AGC), 1 and 16–24 h following transfusion were $17.5 \pm 16.5 \times 10^9$/L and $12.4 \pm 8.1 \times 10^9$/L, respectively (Table 2). Nitroblue tetrazolium test (NBT) described elsewhere (10) was done 16–24 h after the transfusions to evaluate their efficacy. Dye reduction measured by NBT ranged between 14 and 46%, while it was totally negative previously.

DISCUSSION

Despite adequate antifungal prophylaxis, invasive Aspergillosis is now, by far, the most common cause of death, accounting for about one third to one half of the mortality in CGD.

Aspergillus, predominantly *fumigatus* is the most commonly implicated fungus in CGD (11). Despite

amphotericin, usually given for several months against the risk of resurgence of persisting microorganisms, mortality still remains around 30% due to progression and dissemination into various organ systems such as bones and vertebral bodies with subsequent spinal paralysis (9). In case of invasive Aspergillosis, recent conventional treatments consist of a high dose amphotericin B, surgery and granulocyte transfusions (11). Recently, new antifungal agents such as second generation triazoles (e.g. voriconazole, posaconazole), caspofungin, and micafungin (FK463) and new echinocandin's have been reported to be effective in immunocompromised patients with *Aspergillus* infections, including those who proved unresponsive to conventional therapies (12–14).

Only a small series of patients treated with granulocyte transfusions therapies for fungal infections have been reported to date (15). On the other hand, several encouraging case reports and pilot series of granulocyte transfusion therapies, where G-CSF mobilized granulocytes were used for fungal infections have been recently published (9,16–18).

Here, we report an initial attempt and results obtained from the G-CSF + dexamethasone protocol for donors, and details of the apheresis procedure in generating reasonable amounts of functioning granulocytes using the Fresenius AS.TEC system.

Price et al. reported that the combination of G-CSF (600 μg s.c. in a single dose) plus dexamethasone (8 mg orally in a single dose) allows collection of approximately 80×10^9 granulocytes (1). Mobilization of granulocytes was greater after the administration of G-CSF (600 μg) and dexamethasone (8 mg); the granulocyte count increased >12-fold from a mean baseline value of 3554/μL to 43 017/μL at 12 h (4). However, a single dose G-CSF regimen of 450 μg was recently shown to be as effective as 600 μg in the mobilization of granulocytes within 12 h in healthy individuals (19). Moreover, the use of a lower dose of G-CSF for donor mobilization created significant cost savings without compromising the yield of granulocyte in the product (4).

The AGC of granulocyte concentrates in the 20 leukapheresis products of our patients varied between 6.4 and 36.6×10^9 with 450 μg of G-CSF + 8 mg of dexamethasone. To prevent a premature onset of apoptosis that can be triggered by isolation and irradiation, as indicated by Özşahin et al. (9), we administered G-CSF (5 μg/kg/day, s.c.) to our patients, before granulocyte transfusion. The transfusion of $20.7 \pm 10.8 \times 10^9$/L granulocytes (mean ± SD) increased the mean AGC of the patients from $9.7 \pm 6.3 \times 10^9$/L to $17.5 \pm 16.5 \times 10^9$/L at 1 h following the transfusion. The transfused granulocytes persisted in the circulation, as reflected by a mean AGC of $12.4 \pm 8.1 \times 10^9$/L at the following morning. We also found that granulocytes collected from normal donors, stimulated with a combination regimen, have near normal function in vitro when measured by NBT. Our results suggest that transfusion of granulocytes every other day could supply adequate amount of functioning granulocytes to CGD patients.

Granulocyte transfusions are associated with a number of potential adverse reactions, including fever, chills, hives, respiratory distress, or hypotension (1,2). Transfusion related acute lung injury (TRALI) can also be seen in alloimmunized recipients (20). In our study, no severe adverse reaction other than a transient decrease in oxygen saturation in the second patient was encountered during the course of 20 transfusions. However, it resolved spontaneously within a few minutes and seems to be related to the chronic ongoing lung problems of the patient.

Finally, granulocyte transfusions from G-CSF plus dexamethasone stimulated donors seem to be a choice of treatment in CGD patients, especially for those where bone marrow transplantation is not an option.

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