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Functions of Granulocytes After Allogeneic Bone Marrow Transplantation*

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Summary. The phagocytosis and intracellular killing by granulocytes as well as the opsonizing capacity of the serum were studied in 13 patients who had undergone allogeneic bone marrow transplantation. Phagocytosis was normal in all patients. A moderately impaired opsonic activity of the serum was found in two patients, who were investigated within 30 days after the transplantation. The intracellular killing was less than control values in two patients. In one patient this was probably due to the existence of a split chimerism.

Key words: Phagocytosis – Intracellular killing – Granulocytes – Opsonins – Bone marrow transplantation

Introduction

Granulocytes are of great importance for the host defence against bacterial infections. These cells phagocytose the invading micro-organisms, facilitated by opsonins (antibodies and complement component C3b), and then kill them intracellularly. Therefore, after bone marrow transplantation, not only the number of granulocytes in the blood is important but also the functional capacity of these phagocytic cells.

To gain more insight into this aspect of recovery of the host defence system after bone marrow transplantation, we investigated the phagocytosis and intracellular killing by granulocytes as well as the opsonizing capacity of the serum in 13 patients who had undergone bone marrow transplantation. For this study, a method was used by which the rates of phagocytosis and intracellular killing can be measured independently [2].

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Patients

Eight of the 13 patients had received an allogeneic bone marrow transplant for aplastic anaemia and the other five for acute leukaemia. Pre-transplant treatment in five of the eight patients with aplastic anaemia consisted of cyclophosphamide and total lymphoid irradiation. One of the other three patients was treated with cyclophosphamide and total body irradiation, one with cyclophosphamide alone, and one with cyclophosphamide, procarbazine, and anti-lymphocyte globulin. Of the five patients transplanted for acute leukaemia, three were treated before transplantation with cyclophosphamide and total body irradiation, one with daunorubicin, procarbazine, cyclophosphamide, anti-lymphocyte globulin, and total body irradiation, and one with cytosine arabinoside, 6-thioguanine, daunorubicin, cyclophosphamide, and total body irradiation. The age of the patients ranged from 13 to 37 years. All patients were investigated soon after they achieved complete haematological reconstitution ($> 1,000$ granulocytes/ mm^3), which in all but one patient occurred within 60 days after transplantation. Eight patients were investigated within 36 days after the transplantation, four patients within the next 30 days, and one patient 200 days after bone marrow transplantation. None of the patients had acute graft-versus-host disease or a manifest infection at the time of investigation. Most of the patients grafted for aplastic anaemia were treated with partial antibiotic decontamination (PAD) [3], and were taking glucocorticosteroids and methotrexate once a week when the granulocyte functions were assayed. One patient was given anti-thymocyte globulin on the day before the investigation and one patient was receiving plasma infusions twice a week. Of the patients grafted for acute leukaemia, two were treated with PAD, two with glucocorticosteroids, one with ethambutol, isoniazide, and amphotericin B, one with plasma infusions twice a week, and one with cotrimoxazole and miconazole, which were stopped on the day of the investigation.

Materials and Methods

Isolation of Granulocytes and Preparation of Serum

Granulocytes were isolated from heparinized blood by dextran sedimentation of the erythrocytes after which they were washed and re-suspended to a concentration of $10^7/\text{ml}$ in Hanks' balanced salt solution. Serum was prepared, after clotting of blood for 1 h at room temperature, by 20 min centrifugation at $1,100 \times g$, and was stored at -20°C . Most of the opsonic activities were assayed directly after preparation of the serum.

Phagocytosis

Equal volumes of 10^7 granulocytes/ml and 10^7 *Staphylococcus aureus*/ml were incubated at 37°C under rotation (4 rpm) in the presence of 10% (vol/vol) AB serum. At 0, 60, and 120 min, samples were taken, after which the granulocytes were spun down by centrifugation at $110 \times g$ for 4 min and the number of viable bacteria in the supernatant was determined microbiologically. Phagocytosis was expressed as the percentage decrease in the number of viable extracellular *S. aureus* [2].

Opsonization

In the opsonization experiments phagocytosis was assessed with the use of granulocytes from healthy donors and serum of the patients.

Intracellular Killing

Equal volumes of 10^7 granulocytes/ml and 10^7 pre-opsonized *S. aureus* were incubated at 37°C under rotation (4 rpm). After 3 min, phagocytosis was stopped by cooling the tubes in crushed ice. The granulocytes were spun down by centrifugation at $110 \times g$ for 4 min and washed twice to remove the non-phagocytized bacteria. Next, the cells containing ingested bacteria were re-incubated with 10% (vol/vol) AB serum at 37°C under rotation (4 rpm), and samples were taken at 0, 60, and 120 min. The granulocytes were then spun down by centrifugation, and lysed by re-suspending them in distilled water containing 0.01% (wt/vol) bovine albumin.

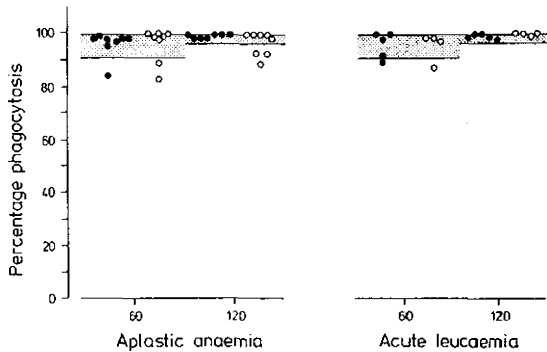


Fig. 1. Phagocytosis by granulocytes (●) and opsonic activity of the patients' serum (○). Each dot represents the findings in one patient. Two patients transplanted for aplastic anaemia showed diminished opsonic activity at 60 and 120 min

The number of viable intracellular bacteria was determined microbiologically, and the decrease in this number was taken as a measure of intracellular killing [2].

Results

Phagocytosis of *S. aureus* by granulocytes in the presence of donor serum was normal in all patients (Fig. 1).

The opsonic activity of the patients' sera towards *S. aureus* was normal, except in the case of two patients transplanted for aplastic anaemia, whose serum gave a moderately impaired ingestion at 60 and 120 min (Fig. 1). The sera of these two patients were investigated 19 and 28 days after bone marrow transplantation. The sera of the other patients were investigated more than 32 days after the transplantation.

The intracellular killing of *S. aureus* was lower than control values in two patients, one transplanted for acute leucaemia and the other for aplastic anaemia. In these cases the time of investigation was 49 and 36 days after transplantation, respectively (Fig. 2). In the patient transplanted for aplastic anaemia, intracellular killing was followed over a period of 3 years and remained depressed. This patient shows signs of split chimerism. The granulocytes of the donor (his brother) showed normal intracellular killing of *S. aureus*.

Discussion

Almost all of the transplanted patients showed normal opsonic activity of the serum as well as normal phagocytosis and intracellular killing by granulocytes when investigated as soon as haematological reconstitution had been attained. A slightly diminished opsonic activity was found for the sera of two patients investigated shortly after transplantation. It is not yet certain whether the opsonic activity of the serum differs with the duration of the interval after transplantation, but since the impairment is only moderate it is unlikely to have clinical importance.

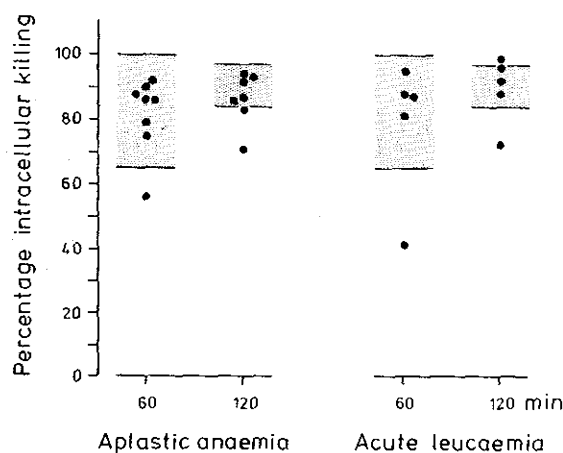


Fig. 2. Intracellular killing by granulocytes. Each dot represents the findings in one patient

The only serious abnormality found was depressed intracellular killing in two patients. In one of them this phenomenon might be explained by a split chimerism, the granulocytes acquired from the donor possibly functioning normally but not his own cells. No explanation can be offered for the decreased intracellular killing in the other patient. Follow-up studies could not be performed because this patient died two days after the investigation.

Territo et al. [5] found normal intracellular killing of *Candida albicans* and *S. aureus* by granulocytes and normal chemotaxis of the granulocytes after allogeneic bone marrow transplantation. These neutrophil functions were not affected by the presence or absence of graft-versus-host disease, the time after transplantation, the disease for which the patient was transplanted, or the pretransplant conditioning. The impaired chemotaxis observed by Clark et al. [1] in patients with graft-versus-host disease was probably related to the treatment with anti-lymphocyte globulin which most of their patients received. Pahwa et al. [4] found decreased chemotaxis of granulocytes and monocytes and decreased intracellular killing of *S. aureus* by granulocytes of some patients investigated within 33 days after bone marrow transplantation; later, all of these functions became normal. On this basis it may be assumed that as soon as granulocytes re-appear in the blood after bone marrow transplantation, they have a normal functional capacity. Decreased host resistance to infections after bone marrow transplantation seems not to be due to granulocyte dysfunction. Deficiencies in cell-mediated immunity are probably of more importance in the infections occurring after bone marrow transplantation.

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