Study of Langerhans Cells After Allogeneic Bone Marrow Transplantation

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We assessed the number of Langerhans cells (LC) before and after bone marrow transplantation (BMT) in 27 patients in order to study the fate and behavior of these dendritic antigen-presenting cells following allogeneic BMT. LC were identified using monoclonal antibody OKT6 on skin biopsies performed on days −10, 0, 11, 25, 39, 120, and 365. In a control group composed of 15 healthy adults aged 20–37 yr, the mean number of LC (±SEM) was 25.6 ± 1.17/0.1 sq mm of epidermal surface. Our study shows that pretransplant, the number of LC in patients with aplastic anemia or leukemia was lower than that of controls. The finding of low numbers of LC in patients with untreated aplastic anemia is suggestive of a medullary origin of LC in man. Moreover, during the early posttransplant period, nearly all patients present a severe deficit in LC. This deficit may delay the maturation of their immune system. The number of LC reaches nearly normal levels 4–12 mo after BMT. Finally, we have noted a significant impairment of LC reconstitution in patients with acute graft-versus-host disease (GVHD), providing evidence that this defect may be an important mechanism involved in acute GVHD-related immunodeficiency.

SOON AFTER BONE MARROW transplantation (BMT) became an established therapy for various immunologic and hematologic diseases, it became apparent that long-lived immunologic memory is not transferred by the marrow inoculum.[1] The engrafted lymphoid system must go through a second round of ontogeny. Consequently, patients are afflicted with a severe combined immunodeficiency, which lasts from 4 to more than 12 mo.[1,2] This profound immune defect is associated with an increased susceptibility to bacterial, fungal, protozoal, and viral infections.[3-8] In vitro studies have demonstrated that the impairment of immunoglobulin production and cell-mediated immunity is complex and multifactorial, affecting all effector functions studied so far for various periods of time.[1,9-14]

Antigen presentation is the major influence that channels immune responsiveness.[15] Indeed, without proper presentation, an antigen may remain immunologically silent or induce specific suppressor cells.[15] Dendritic (or interdigitating) cells appear to be the most effective antigen-presenting cells in mice[16] and man.[17,18] From their strategic location in the thymic medulla and peripheral lymphoid tissues, they contribute to T cell education and may influence the structure of the T cell repertoire.[19,20] Langerhans cells (LC) found in the skin are part of the dendritic cell family.[21,22] In mice, studies of skin transplants suggested that LC of the transplanted skin were derived from a mobile pool of recipient cells.[23] Moreover, studies of radiation-induced chimera reconstituted with allogeneic marrow cells demonstrated that LC originate from bone marrow precursor cells.[23] So far, there are no relevant data in regard to the origin of LC in man. The present study was undertaken to evaluate the fate and behavior of LC in man using allogeneic BMT as a working model. Assuming that LC in man also originate from bone marrow precursor cells, LC of recipient origin should disappear following bone marrow aplasia induced by conditioning regimens, to be replaced by LC of donor origin after transplantation. Therefore, transplanted patients could present a transitory deficit in LC that may delay the maturation of their immune system. Also, acute and chronic graft-versus-host disease (GVHD) impair posttransplant immune reconstitution and are associated with an increased risk of infection.[2,3] We were thus interested to see if GVHD alters the reconstitution of LC following bone marrow transplantation. To assess these problems, we have evaluated the number of LC on serial skin biopsies before and after allogeneic BMT in 27 patients. LC were identified using monoclonal antibody OKT6, which is considered to be the most sensitive and specific LC marker.[24-26]

MATERIALS AND METHODS

Study Design

Twenty-seven consecutive patients receiving an allogeneic marrow transplant for severe aplastic anemia or hematologic malignancy were entered into this study. The study design was approved by the Human Subjects Review Committee of l'Hôpital Maisonneuve-Rosemont. All patients and their donors were HLA-A, B, and C identical siblings, who were mutually nonreactive in mixed leukocyte culture. All aplastic patients fulfilled the criteria for severe aplastic anemia.[27] One of these patients had a posthepatitic aplasia [unique patient number (UPN) 1], one followed congenital amegakaryocytic thrombocytopenia (UPN 7), and 9 were idiopathic. Untransfused patients with aplastic anemia were conditioned with cyclophosphamide alone[28] (UPN 1 and 7), while those with

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prior transfusions were also given buffy coat cells[29] (UPN 4 and 5), total body irradiation[30] (UPN 12 and 27), or total lymphoid irradiation[31] (UPN 9, 10, 15, 17, and 21). None of the patients with aplastic anemia had received androgens, steroids, antithymocyte globulin, or any other type of therapy prior to transplantation. With two exceptions, patients with acute leukemia or chronic myelogenous leukemia were conditioned with cyclophosphamide and total body irradiation.[32] UPN 11 received cyclophosphamide, busulfan, and total body irradiation, and UPN 14 was conditioned with busulfan and cyclophosphamide.[33] To prevent acute graft-versus-host disease (GVHD), methotrexate was administered to all patients, following the Seattle schedule.[32] The severity of acute GVHD was evaluated by grading of skin, liver, and gastrointestinal tract involvement according to the clinical and histologic criteria of Thomas et al.[32] Briefly, clinical grade I acute GVHD refers to a skin rash affecting less than 50% of the body surface, grade II to mild liver and/or gut involvement with a mild to generalized skin rash, and grade III–IV refers to moderate to severe involvement of the gut and/or liver with or without generalized erythroderma. Histologic staging of skin involvement is based on a scale ranging from 1 to 4: grade 1, basal vacuolar degeneration or necrosis; grade 2, grade 1 changes and spongiosis, dyskeratosis, or eosinophilic necrosis of epidermal cells; grade 3, focal microscopic dermal-epidermal separation; grade 4, frank loss of epidermis. Patients with acute GVHD clinical grades II–IV were treated with methylprednisolone 5–20 mg/kg/day. Diagnostic criteria for chronic GVHD were those of Sullivan et al., and treatment consisted of prednisone, 1 mg/kg q.o.d., and azathioprine, 1.5 mg/kg daily.[34]

Laboratory and Statistical Methods

Punch skin biopsies (4 mm) of the arms were obtained on days −10, 0 (day of transplant), 11, 25, 39, 120, and 365. Because of logistic considerations, it has been impossible to perform skin biopsies in all patients on each of these days. The control group consisted of 15 healthy adults, aged 20–37 yr. Skin specimens were immediately sectioned into two parts. One part was fixed in 10% buffered formalin, embedded in paraffin, stained with hematoxylin-eosin, and examined for signs of acute GVHD. Grade 2 histologic changes[32] were required for diagnosis of acute GVHD. The second part was used to quantify LC. It was snap frozen in liquid nitrogen and maintained at −80°C until use. Unfixed cryostat sections (4 μm) were incubated for 30 min with anti-T6 antibody (Ortho Pharmaceuticals, Raritan, NJ, lot no. 11M047), diluted 1:20 in phosphate-buffered saline (PBS), and then incubated for 30 min with fluorescein-conjugated goat anti-mouse IgG (Meloy Laboratories, Springfield, VA, lot no. 29995) diluted 1:40. Sections were mounted on coverslips with 10% PBS in glycerin and examined under a Leitz Dialux 20 fluorescence microscope. The thickness of epithelial cell layers (stratum malpighii plus stratum granulosum) in our biopsy specimens is 0.05 mm and did not show significant variation from subject to subject. LC were counted in skin sections of 2-mm length, and areas containing hair follicles were omitted. The results were expressed as the number of OKT6-positive cells/0.1 sq mm (0.05 mm × 2 mm) of epidermal surface. The statistical significance of the difference between means was calculated with the t test.

RESULTS

The mean number of LC in the control group was 25.6 ± 1.17 (SEM)/0.1 sq mm of epidermal surface. Before transplantation, the number of LC was lower in patients than in controls ($p < 0.001$). This difference is significant both for patients with leukemia and for those with severe aplastic anemia (Table 1). In the group of aplastic patients studied on day −10, 5 had a moderate to severe LC deficit (0, 1, 9, 11, and 14 LC/0.1 sq mm), and 2 patients had normal numbers of LC (22 and 28 LC/0.1 sq mm). Following administration of the conditioning regimen, the number of LC decreased progressively, to reach a nadir around day 11 (Fig. 1). LC had completely disappeared or reached very low levels in all leukemic patients and in 5 of 7 aplastic patients studied at this time. Two aplastic patients (UPN 17 and 21) still had numerous LC (14 and 25/0.1 sq mm, respectively) on day 11. UPN 17 and 21 were the only aplastic patients that did not present a deficit of LC prior to transplantation. Thereafter, the number of LC steadily increased to attain nearly normal levels between 120 and 365 days after transplantation (Fig. 1).

Table 1. Pretransplant (Day −10) Number of Langerhans Cells/0.1 sq mm

	Number of Subjects	Mean Number of Langerhans Cells (± SEM)	Significance Level
Controls	15	25.6 ± 1.17	
Patients	19	14.5 ± 2.16	$p < 0.001$
Severe aplastic anemia	7	12.1 ± 3.59	$p < 0.001$
Leukemia*	12	15.9 ± 2.62	$p < 0.01$

*Five patients with CML in chronic phase, 4 with acute leukemia in complete remission, and 3 with acute leukemia in relapse.

To evaluate the possible influence of acute GVHD on LC reconstitution, we assessed the number of LC on day 39. Toxic effects of the conditioning regimen, which can significantly interfere with the histologic

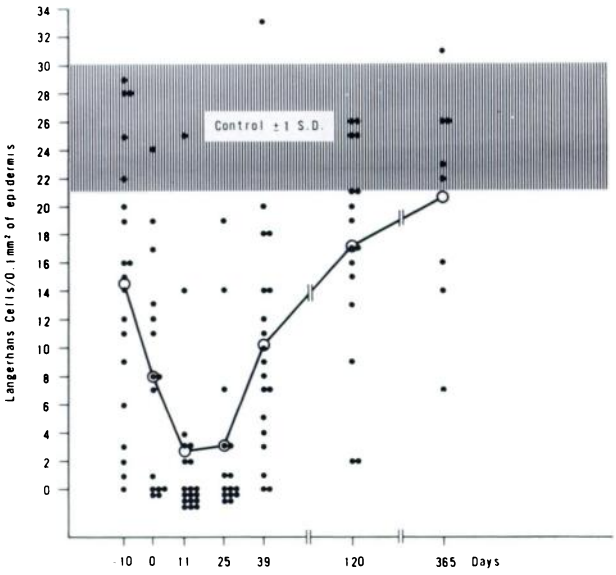

Fig. 1. Number of Langerhans cells per 0.1 sq mm of epidermal surface. Day 0 is the day of marrow infusion. Each dot represents a single patient. (O), mean.

diagnosis of GVHD, have disappeared by this time.[35] In 6 patients with acute GVHD clinical grade II–IV (2 grade II, 2 grade III, 2 grade IV), confirmed by histologic skin changes of grade 2–3, we have found a significant deficit in LC (mean ± SEM, 4.2 ± 1.42/0.1 sq mm) compared to patients (n = 13) with clinical grade 0–I GVHD (mean ± SEM, 13 ± 1.94) ($p < 0.02$). On day 120, in 6 patients with signs of chronic GVHD (mainly oral mucositis, sicca syndrome, and abnormal liver functions tests), there was no difference in the number of LC (mean ± SEM, 17.7 ± 3.37/0.1 sq mm) compared to patients without chronic GVHD (n = 10, mean ± SEM, 17.4 ± 2.43).

DISCUSSION

In mice, epidermal LC are derived from a mobile pool of precursor cells that originate in bone marrow, from which they are continually repopulated.[23] A small fraction of LC may also be capable of in situ proliferation.[36] The finding of low numbers of LC in 5 of 7 previously untreated patients with aplastic anemia studied before transplantation is suggestive of a medullary origin of LC in man. Very recently, we obtained a more straightforward demonstration of the medullary origin of LC as we observed Y body fluorescence in epidermal LC of 2 female recipients of male marrow 120 days posttransplant (Pelletier M, et al., unpublished observation). Further studies are necessary to precisely evaluate the proportion of LC that are of donor origin at different times after marrow transplantation. It is difficult to explain why LC of 2 aplastic patients were in the normal range at day −10, as we have found no difference in their peripheral blood counts, marow cellularity, or duration of clinically recognized pancytopenia compared to aplastic patients with low numbers of LC. It appears relevant in this regard to note that, in radiation-induced bone marrow chimeric mice, Katz reported that 25%–70% of LC were of recipient origin 85 days after chimerization, whereas all spleen and peripheral blood cells were of donor origin.[23] These observations suggest that, in the absence of marrow precursor cells, the pool of epidermal LC may not be depleted for a relatively long period of time, either because these cells have a long lifespan or because in situ proliferation of epidermal LC may compensate for the lack of replenishment by marrow progenitors. Selective persistence of marrow precursors for LC in untreated aplastic patient and mice chimera is another possibility but appears less likely.

The deficit of LC found in leukemic patients prior to transplantation is probably related to chronic maintenance chemotherapy. Inhibition of normal hematopoiesis by leukemic cells may be a contributory factor in patients with acute leukemia in relapse.

Following administration of the conditioning regimen, LC disappear from the skin, reaching a nadir around day 11. The low number of LC observed during the first weeks following BMT may contribute to the profound immunodeficiency that characterizes the early posttransplant period.[1,2] Thereafter, the number of LC progressively increases to reach nearly normal levels 4–12 mo posttransplant. Interestingly, most patients regain near-normal immune reactivity during this interval.[1,2] These observations suggest that repopulation of LC and other antigen-presenting cells may be a prerequisite for immune reconstitution.

Acute GVHD is a major cause of morbidity and mortality following allogeneic BMT. Most of these deaths are related to supervening infections, the most serious of all being interstitial pneumonia.[37,38] Many factors may contribute to this increased susceptibility to infections: loss of integrity of skin and mucosal barriers,[38] defective neutrophil chemotaxis,[39] impaired cell-mediated immunity,[13] and treatment of acute GVHD with immunosuppressive agents, such as steroids, antithymocyte globulin, and cyclosporin. The mechanisms responsible for the impairment of cell-mediated immunity during acute GVHD are not well understood. At day 39, we found that patients with acute GVHD grade II–IV have a significantly lower number of LC compared to patients with acute GVHD grade 0–I, providing evidence that deficiency in antigen-presenting cells may play a role in acute GVHD-related immunodeficiency. The cause of the quantitative defect of LC in acute GVHD is obscure, although three hypotheses can be proposed. First, grade II–IV acute GVHD is frequently associated with marrow hypoplasia, which may impair the generation of LC progenitor cells of donor (marrow) origin. Second, at day 39, residual recipient LC with the ability to proliferate in the epidermis may represent a significant proportion of the total pool of LC. These recipient LC could have a role in initiating acute GVHD and could be important targets for the effector arm of the allogeneic attack,[40] which might cause selective depletion of these cells. This hypothesis appears less attractive given the complete disappearance of recipient LC in most patients on day 11. Finally, high-dose methylprednisolone, used to treat acute GVHD, may contribute to delayed LC reconstitution.

Chronic GVHD is also associated with an increased susceptibility to infections.[3] At day 120, we did not find any relation between the number of LC and the presence or absence of chronic GVHD. Mori et al. recently suggested that the impairment of cell-mediated immunity in acute and chronic GVHD may have different pathogeneses.[13] Both processes are associated with an impairment of cell-mediated lympholy-

sis (CML). Addition of interleukin 2 to mixed lymphocyte culture during the sensitization phase restored CML activity to normal levels in patients with acute GVHD, but not in those with chronic GVHD.[13] Our results suggest that low numbers of antigen-presenting cells may contribute to the defective cell-mediated immunity found during acute GVHD, but cannot account for such a defect in chronic GVHD.

In conclusion, our study demonstrates that, during the early posttransplant period, most patients present a severe deficit in LC that may delay the maturation of their immune system. Acute but not chronic GVHD is associated with a significant impairment of LC reconstitution following allogeneic BMT. One should be cautious, however, in extrapolating our data to dendritic antigen-presenting cells in other organs (e.g., thymus, spleen, lymph nodes), as their behavior and properties may not be identical to those of LC. We are currently studying sex-mismatched transplants to evaluate the proportion of LC that are of donor and recipient origin at different intervals following transplantation. These observations should provide some insight into the kinetics and turnover of LC and their role in the immunobiology of bone marrow transplantation.

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