Long-Term Remission From Acute Myelogenous Leukemia After Bone Marrow Transplantation and Recovery From Acute Graft-Versus-Host Reaction and Prolonged Immunoincompetence


A 19-yr-old boy has been in continuous complete remission from acute myelogenous leukemia for 3 yr after allogeneic bone marrow transplantation prepared with combination chemotherapy. During the first year post-transplant, however, the patient developed near-fatal graft-versus-host reaction followed by 11 severe viral and bacterial infections. Immune evaluation during this period revealed multiple defects which were not present prior to transplantation, nor present in the transplant donor: diminution of lymphoid tissue, decline of all immunoglobulin subtypes, deletion of secretory immunoglobulin, disappearance of isohemagglutinins, loss of antibody to diphtheria and tetanus toxoids, cessation of cutaneous hypersensitivity to mumps antigen, and inhibition of serum opsonizing activity. The patient was also unable to develop normal humoral or cellular reactivity to brucella antigen, keyhole limpet hemocyanin, or dinitrochlorobenzene. This patient's course illustrates the severity and chronicity of immunoincompetence associated with allogeneic marrow grafting, the importance of early detection and rigorous treatment of infectious disease in these patients, and the need for improved immunologic reconstitution in human marrow transplantation. It also indicates that complete recovery from the immune defects is possible, and that long-term remission from acute myelogenous leukemia can be achieved with allogeneic marrow transplantation.

A LLOGENEIC BONE MARROW transplantation in patients with acute leukemia and aplastic anemia has frequently been complicated by a prolonged immunoincompetent state and an associated susceptibility to severe infection.1,2 Studies by Fass et al.1 and Halterman et al.3 have implicated certain defects in humoral (B-cell) and cellular (T-cell) immunity as responsible for the immunoincompetence, but additional testing is needed to more fully characterize the immunologic derangements in these patients. This report describes a spectrum of immunologic deficiencies in a patient successfully engrafted with allogeneic bone marrow for acute myelogenous leukemia (AML).

MATERIALS AND METHODS

The immunologic methods utilized in this study have been described in detail elsewhere: mixed-lymphocyte culture (MLC) and phytohemagglutinin (PHA) lymphocyte blastogenesis (performed by Dr. B. Leventhal),2 spontaneous human lymphocyte rosette formation with sheep red blood...
cells (performed by Dr. C. Olweny),\textsuperscript{4} dinitrochlorobenzene (DNCB) skin testing (performed by Dr. P. Chretien),\textsuperscript{5} intradermal skin testing (performed by Dr. R. Halterman,\textsuperscript{2} quantitative immunoglobulins (performed by Dr. R. Woods and Dr. T. Waldmann),\textsuperscript{6} jejunal immunoglobulin A synthesis (performed by Dr. W. Strober),\textsuperscript{7} and titers of antibodies towards viral antigens (performed by Dr. R. Chambers and Dr. D. Fuccillo),\textsuperscript{8} bacterial antigens,\textsuperscript{9} and keyhold limpet hemocyanin (KLH).\textsuperscript{9}

Lymphocyte stimulation ratios of $\geq 20$ and $\geq 2$ were considered normal for PHA and allogeneic lymphocytes, respectively. Reactivity to intradermal injections of soluble skin tests antigens were considered positive if induration exceeded 5-mm diameter at 48 hr. DNCB skin tests were interpreted as negative, + if erythema, + + if erythema and induration, + + + if vesiculation, and + + + + if vesiculation and bullae. The normal adult range for serum IgG is 550-1770 mg/lOO ml, 40-320 mg/100 ml for serum IgA, 45-155 mg/lOO ml for serum IgM, and 15-742 mg/ml for serum IgE. Antibody to erythrocyte antigen was measured by M. McGinniss, and immunoglobulin allotyping was performed by Dr. A. Steinberg.

Phagocytosis was measured by the amount of radiolabeled heat-killed coagulase-positive \textit{Staphylococcus aureus} associated with normal blood type O granulocytes after 20 min of tumbling at 37°C in 10\textsuperscript{9} serum.\textsuperscript{10} The ability of patient serum to promote phagocytosis by blood type O granulocytes (serum opsonizing activity) was compared with the serum opsonizing activity of normal AB serum. Bactericidal activity was expressed as the percentage of \textit{S. aureus} organisms killed after tumbling at 37°C in 10\textsuperscript{9} serum for 120 min.\textsuperscript{10}

Serum chemotactic generating activity was performed by Dr. E. Patten, utilizing the Boyden Chamber technique,\textsuperscript{11} endotoxin for activation, and granulocytes prepared as described above from blood type O donors to whom the patient was leukoagglutinin negative. Rheumatoid factor was quantitated by the bentonite flocculation titer. Skin biopsy was confirmed to demonstrate GvHR by Dr. G. R. F. Krüger.\textsuperscript{12}

CASE REPORT

Bone marrow transplantation was performed in a 17-yr-old male with AML of 3 yr duration, during which infectious diseases were limited to superficial staphylococcal infections occurring in association with severe neutropenia. At the time of transplantation, his disease was in progressive relapse and resistant to conventional chemotherapy, including combinations of cytosine arabinoside, daunorubicin, vincristine, prednisolone, methotrexate, and 6-mercaptopurine. The donor was an ABO incompatible (donor, type A; recipient, type O) but HL-A genotypically indentical and MLC nonreactive 14-yr-old brother. Pretransplant immunosuppression and tumor ablation was accomplished with a four-drug combination (B.A.C.T.),\textsuperscript{13} which incorporates 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU), 200 mg/sq m, 24 hr prior to bone marrow infusion; cytosine arabinoside, 100 mg/sq m, every 12 hr for $3\frac{1}{2}$ days; cyclophosphamide, 45 mg/kg/day for 4 days; and 6-thioguanine, 100 mg/sq m, every 12 hr for 3$\frac{1}{2}$ days prior to transplantation. No drugs are administered for 24-30 hr preceding bone marrow infusion. During this immunosuppressive therapy, preformed anti-A isohemagglutinins were reduced in titer from 1:128 to 1:2 with intravenous infusions of Witebsky group A substance and type A\textsubscript{1} red blood cells.\textsuperscript{13} One day after completion of the B.A.C.T. regimen, the patient received $5.2 \times 10^8$ bone marrow cells per kg body weight from the transplant donor, utilizing the technique of Thomas et al.\textsuperscript{14} Full hematologic recovery and remission from AML were noted within 3 wk after transplantation, the details of which have been published elsewhere.\textsuperscript{13}

\textit{Graft-Versus-Host Reaction (GvHR)}

GvHR began 29 days post-transplant and was characterized by marked diarrhea, thrombocytopenia, hepatitis, and a skin rash histologically confirmed as GvHR. The enteropathy was nearly fatal, having been complicated by intestinal fluid losses averaging 10 liters daily for 2 wk, and by an actue intraabdominal hemorrhage of approximately 2000 cc. Treatment of GvHR in this patient has been reported elsewhere.\textsuperscript{13} It included administration of antilymphocyte serum (supplied by Dr. G. Mathé), 10 cc intravenously daily from day 39 to day 45, passively administered “blocking antibody” (obtained from the patient’s mother), parenteral alimentation, isolation in a laminar air flow room, oral nonabsorbable antibiotics, and intravenous broad-spectrum anti-
biotics. On this regimen, the signs and symptoms of acute GvHR gradually diminished and subsided 60 days post-transplant.

**Viral and Fungal Infections**

Recurrent infections began 2 mo after clinical recovery from GvHR and persisted until 10 mo post-transplant (Fig. 1). Oral candidiasis developed during GvHR and did not clear until 110 days post-transplant despite topical therapy and discontinuation of broad-spectrum antibiotics 44 days post-transplant. Multiple viral infections occurred between 85 and 275 days post-transplant, as implicated by the clinical illnesses, virus cultures, and antiviral antibody increments (Fig. 1). Those requiring hospitalization were influenza B viremia and varicella-zoster. The latter occurred as Ramsay-Hunt syndrome with generalized lesions and cleared slowly despite 4 days of therapy with cytosine arabinoside. Subsequent to 10 mo post-transplant, viral illnesses have been mild, infrequent, and limited to the upper respiratory tract.

**Bacterial Infections**

Between 117 and 300 days post-transplant, the patient also sustained multiple bacterial infections (Fig. 1). These included pneumococcal pneumonia with bacteremia, two episodes of acute purulent pansinusitis, several bouts of acute otitis media, and one occurrence each of staphylococcal aureus tympanomastoiditis and Corynebacterium carbunculosis. Since 10 months post-transplant the patient has had no known bacterial infections, and he has been able to undergo tympanoplasty, skin allografting, lymph node biopsy, small bowel biopsy, and bilateral maxillary antrectomies without difficulty.
RESULTS

Evidence for Engraftment

Hematopoietic engraftment was evidenced by rapid marrow restoration of myeloid, erythroid, and megakaryocytic elements, and by eventual complete replacement of recipient red cells with those of donor type. B-cell engraftment was indicated by total conversion of immunoglobulins to those of donor allo-type. T-cell engraftment was suggested by transfer from donor to recipient of cutaneous delayed hypersensitivity to brucella, poison oak, and poison ivy, by acceptance of skin allograft from the donor simultaneously with primary rejection of third-party skin, and by the development of acute GvHR. To date, there has been no evidence of marrow graft rejection or of mixed chimerism. However, multiple immunologic deficiencies accompanied marrow engraftment and persisted for nearly 1 yr after transplantation.

T-Cell Dysfunction (Fig. 2)

Lymphopenia was noted only during GvHR, but on physical examination generalized lymphoid hypoplasia was prominent throughout the first year post-transplant. For example, the lymphoid tissue of Waldeyer's Ring disappeared.
during GvHR, and no lymph nodes were palpable until 10 mo post-transplant. At that time, three nodes were detected in the left posterior cervical region (efferent to the patient's zoster), and biopsy of one of these revealed normal morphology. Two months post-transplant and after treatment with antilymphocyte serum, none of the circulating lymphocytes could be demonstrated as T-cells by the technique of spontaneous rosette formation with sheep red blood cells, whereas 10 mo after transplantation approximately one-half of the circulating lymphocytes formed rosettes. In vitro lymphocyte blastogenesis to PHA was normal before and after GvHR, but depressed on three occasions during GvHR (Fig. 2). Lymphocyte reactivity to random allogeneic leukocytes in MLC has been normal since transplantation.

Following transplantation, the patient lost previously established cutaneous reactivity to mumps and to brucella, the latter of which had been conferred from the donor. Reactivity to DNCB was not transferred from the DNCB-positive donor, nor later induced by attempts at direct sensitization (Fig. 2) until 18 mo post-transplant, when sensitization elicited a ++++ reaction. The patient also failed to develop a hypersensitive reaction to KLH despite multiple attempts at sensitization (Fig. 2). He was also negative to streptokinase-streptodornase, PPD, trichophyton, and histoplasma antigens on many occasions, both before and after engraftment. Eleven months post-transplant, delayed cutaneous hypersensitivity toward diphtheria and tetanus toxoids was demonstrated after immunization with these antigens, and the candida skin tests converted to positive after recovery from oral moniliasis. At 10 mo post-transplant, the patient was able to reject a random donor skin allograft within 12–15 days, concomitantly with development of circulating cytotoxic lymphocytes directed specifically and only toward target lymphocytes from the third-party skin donor.

In summary, T-cell dysfunction was characterized primarily by loss of previously established delayed hypersensitivity to microbial and viral antigens, and by failure to develop delayed hypersensitivity to new test antigens. It was accompanied by generalized lymphoid aplasia and was most prominent between 3 and 10 mo post-transplant, during which the patient sustained multiple viral infections, as well as oral candidiasis.

B-Cell Dysfunction (Fig. 3)

Serum levels of all immunoglobulin subtypes fell after transplantation. Decreases in serum IgG and IgM were transient, but depression of the other subtypes persisted for 12 (IgD and IgE)–15 (IgA) mo. IgA deficiency was most marked, with no detectable serum or salivary IgA and negligible in vitro synthesis of IgA by jejunal mucosa obtained via small bowel biopsy 7 mo post-transplant. Plasma cell number was significantly diminished in the lamina propria of the small intestine, but not in bone marrow aspirates or cervical lymph node biopsy. Repeat small bowel biopsy 15 mo post-transplant revealed normal plasma cell number and restoration of IgA synthesis.

Loss of specific antibody was also noted, including disappearance of several antibodies ordinarily associated with lifelong serum titers. Anti-B isohemagglutinin dropped from 1:128 post-GvHR to zero titer 8 mo later. Over a 5-mo
Fig. 3. B-cell dysfunction during the first year post-transplant. Bars on immunoglobulin ordinates represent normal ranges. Serum and secretory IgA returned to normal 15 mo post-transplant.
period, circulating antibodies to diphtheria and tetanus toxoids fell from titers of 1:4096 to undetectable levels. The patient had received tetanus toxoid 1 yr prior to transplantation, and both the patient and his marrow donor had been fully vaccinated with diphtheria toxoid, including booster doses, during childhood. Other antibodies which disappeared post-transplant included complement-fixing antibodies to parainfluenza 1, parainfluenza 3, and respiratory syncytial viruses.

Antibody production in response to certain specific antigenic challenges was also impaired. Most striking were the serum antibody responses to diphtheria and tetanus toxoids (Fig. 3), to which the patient had been previously immunized. Administration of these antigens 7 mo post-transplant was associated with a markedly attenuated antibody rise, resembling that of a weak primary response to first exposure, and with a positive Schick test. When immunization was repeated 2 mo later, however, secondary-type antibody increments were observed, and the Schick reaction was neutralized.

Similarly, serum anti-KLH antibody was not detected until the fourth immunizing attempt 11 mo post-transplant (Fig. 3). Attenuated responses to brucella antigen were also noted at 10 and 11 mo post-transplant. Antiviral antibody responses associated with specific infections appeared to be entirely normal (Fig. 1).

In summary, B-cell dysfunction involved generalized immunoglobulin deficiency, deletion of pre-existing circulating antibody, and impaired antibody production to specific test antigens. Most of these deficits resolved by 10 mo post-transplant, after which bacterial infections subsided and viral diseases markedly improved.

**Opsonic Dysfunction (Fig. 4)**

Studies of granulocyte function and serum opsonins were prompted by the frequent recurrence of bacterial infections in the presence of normal numbers of circulating granulocytes. In five separate experiments performed with patient granulocytes between 147 and 292 days post-transplant, the phagocytic uptake (mean ± SE) of the *S. aureus* inoculum was 55% ± 2% using patient serum and 71% ± 3% using control AB serum (*p* < 0.001, Student’s t test), and the killing was 49% ± 2% of the bacterial inoculum using patient serum and 92.5% ± 1.4% using control AB serum (*p* < 0.001). Attenuation of phagocytic and bactericidal activity was also observed with granulocytes obtained from the bone marrow donor and from random, unrelated blood type O donors. Patient serum obtained prior to transplantation, and since 347 days post-transplant, demonstrated normal opsonic activity (Fig. 4).

Presence of an inhibitor, rather than of a deficiency, of opsonins in patient serum was suggested when the phagocytic studies were performed with mixtures of control and patient serum. Opsonic activity of all mixtures tested did not differ significantly from that of patient serum alone (Table 1). When the bacteria were preopsonized in 10% serum and washed prior to being added to a serum-free cell suspension, the patient’s serum was still less efficient than control serum in promoting phagocytosis (Table 1), suggesting that the inhibitor affected an opsonization process rather than directly inhibiting granulocyte
Fig. 4. Opsonic dysfunction during the first year post-transplant. Serum opsonizing activity represents the difference of patient serum and normal AB serum in promoting the phagocytosis of S. aureus organisms by normal blood type O neutrophils during 20 min of tumbling at 37°C in 10% serum.

Table 1. Phagocytosis of S. aureus by Blood Group Type O Granulocytes in Mixtures of Patient and Control AB Serum—A Representative Experiment. Similar Results With Patient Serum were Obtained on Days 147, 194, 235, 252, and 269 Post-transplant

<table>
<thead>
<tr>
<th>Serum Added</th>
<th>Per cent Uptake at 20 min*</th>
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<tr>
<td>Serum-free control</td>
<td>6</td>
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<tr>
<td>5% AB</td>
<td>71</td>
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<td>5% patient</td>
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<td>10% AB</td>
<td>73</td>
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<tr>
<td>10% patient</td>
<td>57</td>
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<tr>
<td>10% AB (heated)†</td>
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<td>10% patient (heated)†</td>
<td>9</td>
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<td>5% AB + 5% patient</td>
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<td>5% AB + 15% patient</td>
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<td>5% AB (heated) + 5% patient</td>
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<tr>
<td>Preopsonized with AB serum</td>
<td>81</td>
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<tr>
<td>Preopsonized with patient serum</td>
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*Per cent of bacteria associated with normal blood type O granulocytes. Each value represents the mean of triplicate samples.
†Heated at 56°C for 30 min.
phagocytosis. Heat treatment of patient serum yielded the same opsonic activity as heat-treated normal serum (Table 1), indicating that the patient's opsonic abnormality was related primarily to a disturbance of heat labile opsonic component(s).

With evidence that the inhibitor was directed against a heat labile opsonin(s), studies of complement-mediated functions were undertaken. The patient's serum was unable to generate normal amounts of endotoxin-activated chemotactic activity on days 194, 216, 235, and 257 (Fig. 4), the number of neutrophils migrating toward patient's serum ranging from 23% to 39% of the number of cells migrating toward normal serum. Also, on days 276, 297, and 312 the patient's serum contained anticomplementary activity which interfered with routine serologic testing for complement-fixing antibodies (Fig. 4). That is, the patient's serum in the antigen-free controls inhibited the hemolytic activity of guinea pig complement used in these assays. Serum from the bone marrow donor was devoid of antiopsonic activity or anticomplementary activity and demonstrated normal chemotactic activity on all occasions tested.

DISCUSSION

The patient presented is the longest surviving recipient of allogeneic sibling bone marrow for the treatment of acute myelogenous leukemia. He has been in continuous unmaintained complete remission for 3 yr, and is employed full time. That he has had no infections for 2 yr indicates that it is possible to recover completely from the immunoincompetence associated with allogeneic marrow grafting. His course also demonstrates that long-term remission from acute leukemia can also be achieved with marrow grafting prepared with combination chemotherapy, as well as with preparation by supralethal whole-body irradiations.¹

During the first year post-transplant, however, the patient sustained 11 infectious illnesses requiring five hospitalizations for medical or surgical intervention. Immunologically, defects in T-cell, B-cell, and opsonic function were demonstrated during this period, which as a composite could readily have accounted for the patient's marked susceptibility to viral and bacterial infections. Evidence for prolonged immunoincompetence following successful allogeneic marrow grafting has been observed in a number of other patients. Of ten patients with acute leukemia and aplastic anemia studied 5–30 mo post-transplant by Fass et al.,¹ none demonstrated normal antibody responses to bacteriophage, and only one could be sensitized to DNCB. Five of these patients developed varicella-zoster infections, and six sustained bouts of pneumonitis between 3 and 11 mo post-transplant. One patient developed *Hemophilus influenzae* bronchitis 14 mo post-transplant, and one overcame a near fatal episode of *H. influenzae* meningitis 17 mo post-transplant. One patient described by Mathé succumbed to varicella-zoster encephalitis 20 mo post-transplant.¹⁵ None of these patients had evidence for graft rejection or leukemic relapse.

The pathogenesis of the immunoincompetence is unclear, principally because a number of immunosuppressive processes can be implicated: (1) endogenous immune defects existing prior to transplantation, (2) the immunosuppressive preparation for grafting, (3) immune defects transmitted from the donor, (4) the
postgrafting chemotherapy administered to prevent GvHR, (5) GvHR itself, (6) the treatment of GvHR (e.g., antilymphocyte serum), (7) incomplete immune reconstitution by the graft, and (8) the effect of infection on immunocompetence. In our patient several of these explanations are unlikely. Since all aspects of the donor’s immune function were normal, it is very unlikely that the deficiencies observed in the patient resulted from defects transmitted directly with the graft. There is little in the history of the recipient to suggest an underlying immunodeficiency state prior to the onset of leukemia, and no abnormalities were demonstrated among those immune tests performed pretransplant. Whether the observed defects were due to GvHR, the antilymphocyte serum, the patient’s infections, or delayed immunologic reconstitution of the graft can not as yet be ascertained.

The possibility that GvHR may have contributed to the immunoincompetence must be considered. Experiments in animals have shown that acute GvHR can suppress immune memory and immunologic reactivity to skin allografts, bacterial antigens, bacteriophage, heterologous red cells, and foreign proteins, and that the magnitude of a given defect is directly proportional to the severity of the GvHR (recently reviewed by Elkins). A similar correlation was made by Halterman et al. in their study of four human recipients of allogeneic bone marrow. Two patients with minimal GvHR following transplantation had only diminished lymphocyte blastogenesis to PHA. One patient with moderately severe GvHR had impaired skin test reactivity and lymphocyte blastogenesis, but normal B-cell function. The fourth patient, who died of fatal GvHR with concurrent infection, failed to demonstrate lymphocyte activation by PHA, antibody response to E. coli, or to brucella antigens, or recovery of serum IgG, IgA, and IgM deficiencies. On the other hand, the data reported by Fass et al. do not corroborate this observation. Although their patients with allogeneic grafts had significantly greater immunoincompetence than the patients with syngeneic grafts, immunoincompetence in the allogeneic graft recipients could not be correlated with the severity of GvHR. Additional studies with syngeneic controls and with allogeneic recipients who did not develop GvHR will be needed to delineate the immunosuppressive effects in man of GvHR.

The demonstration of a circulating inhibitor of serum opsonizing activity during the period of infections emphasizes the need to examine factors other than T-cell and B-cell functions. Although the inhibition has not been characterized, the simultaneous occurrence and disappearance in the patient’s serum of rheumatoid factor and the opsonic inhibitor (Fig. 4) suggests that the opsonic inhibitor may have been a rheumatoid factor. Indeed, rheumatoid factor is capable of fixing complement and of inhibiting phagocytosis in a serum-free system, and studies in the laboratory have detected antiopsonic activity in 33% of 38 sera containing rheumatoid factor in comparison to none of ten sera devoid of rheumatoid factor (Dr. J. Bujak, unpublished data). Antinuclear antibody occurring during chronic GvHR has been observed in laboratory animals, but a systematic search for rheumatoid factor postengraftment has not been conducted.
The spectrum of immunologic deficiencies observed in this patient illustrates the severity and prolonged duration of immunoincompetence associated with allogeneic marrow grafting in man. It emphasizes the importance of prevention, surveillance, and vigorous treatment of infections in these patients, and the need for improved immunologic reconstitution following allogeneic marrow transplantation for hematologic malignancy.

REFERENCES

Long-term remission from acute myelogenous leukemia after bone marrow transplantation and recovery from acute graft-versus-host reaction and prolonged immunoincompetence

WA Bleyer, RM Blaese, JS Bujak, GP Herzig and RG Jr Graw