Does Graft-Versus-Host Disease Influence the Tempo of Immunologic Recovery After Allogeneic Human Marrow Transplantation?
An Observation on 56 Long-Term Survivors


This report describes a study of humoral and cellular immune reactivities in 56 patients who have survived for 1 yr to more than 6 yr after marrow transplantation from an HLA-identical sibling for the treatment of aplastic anemia or hematologic malignancy. All were conditioned for the transplant by high doses of cyclophosphamide and/or total body irradiation. Immunologic studies on the marrow donors served to establish the normal range for the tests used. The influence of an episode of graft-versus-host disease (GVHD) on the subsequent immunologic recovery was emphasized. Thirty-two patients had GVHD, which resolved in 19 and evolved into a chronic form in 13. Tests used included serial determinations of serum immunoglobulins, complement (C3 and C4), lymphocyte counts, T and B cells, lymphocyte responses to allogeneic cells and to mitogens, isohemagglutinin titers, clearance of bacteriophage \( \Phi X174 \) (phage) from the blood, primary and secondary antibody responses to phage and to keyhole limpet hemocyanin (KLH), and tests of skin reactivity to recall and neoantigens, dinitrochlorobenzene, and KLH. Concluded from the study were the following: (1) All patients, regardless of whether they had GVHD or not, had pronounced impairment of all immunologic parameters during the first 4 mo after grafting. (2) The speed of immunologic recovery thereafter was faster in patients without GVHD than in those with GVHD. The deficient immune responsiveness in patients with GVHD lasted approximately 2 yr and thereafter tended to persist only in patients with chronic GVHD. A peculiar and unexplained finding in patients with GVHD was significantly higher than normal IgG levels. As a clinical correlate of the prolonged and intense immune deficiency, patients with GVHD showed a tendency toward more frequent and severe infections than those without GVHD. (3) Given enough time, most patients regained near-normal immune reactivity. This occurred much earlier and more frequently in patients without GVHD than in those with GVHD. Patients who regained near-normal immune reactivity did not have unusually frequent infections.

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INCREASING NUMBERS of marrow grafts from HLA-identical siblings have been carried out for the treatment of patients with severe aplastic anemia (AA) or advanced acute leukemia.\(^1,2\) In preparation for the transplant, the patient's own hemopoietic and immunologic systems are inactivated by high doses of cyclophosphamide (CY) and/or total body irradiation (TBI). The subsequent recovery of hemopoiesis and immune defenses is dependent on the proliferation of cells of marrow donor origin. Approximately one-third of patients who achieve a functional marrow graft develop fatal interstitial pneumonias, and half of these have documented evidence of cytomegalovirus infection.\(^3,4\) In addition, a variety of other viral, fungal, bacterial, and parasitic opportunistic infections have been observed during the first few months after transplantation.\(^5\) Infections have been most frequent in patients with graft-versus-host disease (GVHD). The early infectious complications are presumably the result of poor immunologic reactivity.\(^6\) Understanding of the immunologic deficiency and the development of means of its prevention and treatment seem as important as the development of effective methods to treat established infections.

The present report extends a previous observation\(^6\) and describes an evaluation of immunologic reactivity in 56 patients who have survived for at least 1 yr (and up to more than 6 yr) after allogeneic marrow transplantation for the treatment of AA or hematologic malignancy. The influence of an episode of GVHD on the subsequent immunologic recovery will be emphasized. Whenever possible, immunologic studies on the marrow donors served to establish the normal range for each test used.

### MATERIALS AND METHODS

**Patients**

**Description.** Thirty-two patients with AA, 13 with acute myeloblastic leukemia (AML), and 11 with acute lymphoblastic leukemia (ALL) treated with a marrow transplant between July 1970 and October 1975 were studied. Table 1 lists the sex, age, underlying disease, and survival data of the patients studied. Also listed is the severity of GVHD seen after grafting in 30 patients. In 19 patients acute GVHD resolved completely; in 11 it evolved into a chronic form. Two additional patients developed the chronic form without passing through the acute phase.

**Marrow transplantation.** Details on the selection of patients and donors for transplantation,
Table 1. (Continued)

<table>
<thead>
<tr>
<th>Unique Patient No.</th>
<th>Diagnosis</th>
<th>Age/Sex</th>
<th>Acute GVHD (Grade)</th>
<th>Survival (mo After Marrow Transplantation)*</th>
<th>Clinical Condition</th>
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<tr>
<td>270</td>
<td>AA</td>
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<tr>
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<tr>
<td>274</td>
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<td>I</td>
<td>&gt;45</td>
<td>Chronic GVHD: skin, liver</td>
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<tr>
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<td>AA</td>
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<td>II</td>
<td>&gt;45</td>
<td>OK</td>
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<tr>
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<td>0</td>
<td>45</td>
<td>Recurrent leukemia, treated; died</td>
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<td>II</td>
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<td>0</td>
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<td>&gt;33</td>
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<tr>
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<td>21/M</td>
<td>0</td>
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<td>Recurrent leukemia; died</td>
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<tr>
<td>335</td>
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<td>27/F</td>
<td>0</td>
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<td>OK</td>
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<tr>
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<td>I</td>
<td>&gt;27</td>
<td>Recurrent leukemia; died</td>
</tr>
<tr>
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<td>0</td>
<td>&gt;28</td>
<td>OK</td>
</tr>
<tr>
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<td>AML</td>
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<td>362</td>
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<td>15/M</td>
<td>I</td>
<td>15</td>
<td>Recurrent leukemia; died</td>
</tr>
<tr>
<td>365</td>
<td>ALL</td>
<td>4/M</td>
<td>III</td>
<td>&gt;24</td>
<td>Chronic GVHD: skin</td>
</tr>
<tr>
<td>366</td>
<td>AA</td>
<td>34/F</td>
<td>IV</td>
<td>24</td>
<td>Chronic GVHD: skin, liver; died</td>
</tr>
<tr>
<td>369</td>
<td>ALL</td>
<td>19/M</td>
<td>I</td>
<td>&gt;23</td>
<td>Recurrent leukemia</td>
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<tr>
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<td>&gt;22</td>
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<tr>
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<td>10/F</td>
<td>I</td>
<td>14</td>
<td>Recurrent leukemia; died</td>
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<tr>
<td>387</td>
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<td>30/F</td>
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<td>&gt;21</td>
<td>OK; chronic GVHD: skin</td>
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<tr>
<td>394</td>
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<td>12</td>
<td>Chronic GVHD: skin, liver, gut; died</td>
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<tr>
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<td>11</td>
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<td>&gt;17</td>
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<td>520</td>
<td>AA</td>
<td>12/M</td>
<td>III</td>
<td>&gt;16</td>
<td>Chronic GVHD: skin, liver</td>
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<tr>
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<td>&gt;15</td>
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<td>0</td>
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*Survival as of October 1, 1976.
10, no acute GVHD.
Revision of earlier published diagnosis based on newer data.
on the conditioning for transplantation with immunosuppressive agents, on the transplant procedure, and on each patient’s course before and after transplantation were previously described.1,2 Briefly, all patients and their donors were HLA-A and B identical siblings mutually nonreactive in mixed leukocyte culture (MLC). Twenty-four patients with AA were conditioned with CY, 50 mg/kg on each of 4 successive days, one (No. 208) with 1000 rads TBI, and seven with procarbazine alternating with antithymocyte globulin (ATG) in addition to CY.1,3 Eighteen patients with hematologic malignancy were given CY, 60 mg/kg on each of 2 successive days, and then 1000 rads TBI. Some patients were given additional chemotherapy before CY and TBI.2 Three leukemic patients were treated with either TBI alone, daunomycin-TBI, or 1,3-bis(2-chlorethyl)-1-nitrosourea nitrogen mustard-TBI. All patients were given intermittent methotrexate (MTX) within the first 100 days of marrow grafting to prevent or modify GVHD, and 12 (Nos. 209, 264, 294, 321, 365, 377, 386, 394, 512, 520, 536) were treated with ATG for established GVHD.

**Blood genetic markers to prove allogeneic marrow engraftment.** Twenty-one patients had marrow donors of opposite sex. Karyotypic analyses at intervals after grafting revealed exclusively donor-type cells in peripheral blood and bone marrow except in ten patients (Nos. 264, 274, 282, 292, 306, 329, 369, 505, 510, 538) at the time of recurrent leukemia.1,2 Red blood cell antigen and/or enzyme phenotypes were identical with those of the donor after grafting in 14 patients where donor and recipient had documented pregrafting differences and also in patients for whom pregrafting data were not available because of multiple transfusions.1,2,3 Two patients (Nos. 274, 294) permanently accepted skin grafts from the marrow donor transplanted on days 423 and 918 after marrow grafting.

**Consent.** The protocol for this study was subject to approval and annual review by the Human Subjects Review Committees of the University of Washington School of Medicine and the Fred Hutchinson Cancer Research Center. All patients and donors gave consent on forms approved by the Committees to participate in these studies.

**Evaluation of Immunologic Reactivity**

**Serum.** Serum immunoglobulins (IgG, IgM, IgA) were determined quantitatively using radial diffusion.8 Tests for serum complement level (C3 and C4) were kindly carried out by standard techniques in the Central Laboratory, Dept. of Laboratory Medicine, University of Washington.

**Lymphocytes.** Lymphocyte counts were done daily initially and later at frequent intervals. Lymphocytes used in studies of lymphocyte surface markers and function were separated from the peripheral blood by the Ficoll-Hypaque gradient and centrifugation technique described by Boyum.9,10

**Lymphocytes spontaneously forming sheep erythrocyte rosettes (E rosettes).** E rosette forming cells (E-RFC) were determined by the method of Jondal et al.11 At least 200 mononuclear cells were counted to determine the percentage of E-RFC, and the absolute E-RFC count/mm blood was determined on the basis of the lymphocyte count on that day.

**Lymphocytes forming rosettes with antibody-coated erythrocytes in presence of complement (EAC rosettes).** EAC rosette forming cells (EAC-RFC) were determined by the method of Mendes et al.12 using either rabbit anti sheep hemolysin (Difco, Detroit, Mich.) at 1:2000 or rabbit anti sheep red blood cells (SRBC) 19S fraction (Cordis Laboratory, Miami, Fla.) at 1:1000. The percentage and absolute count of EAC-RFC were determined in the manner used for E-RFC, described above.

**Fluorescent staining of surface immunoglobulin (SIg)-bearing cells.** Lymphocytes (3 x 10⁶) were spun at 200 g for 5 min and the supernatant was removed. Cells were resuspended with 0.1 ml polyvalent anti human Ig serum (Meloy, Springfield, Va.) diluted 1:5. This serum was coupled with fluorescein isothiocyanate. Cells were incubated at 4°C for 40 min, then washed three times with Hanks’ balanced salt solution (HBSS). After resuspending the cells with 0.15 ml HBSS, 0.05 ml of the suspension was pipetted onto a slide, covered with a coverslip, and sealed with quick-drying nail polish. The percentage and absolute count of SIg-bearing cells were determined.

**Lymphocyte response to irradiated unrelated lymphocytes.** Lymphocyte response to stimulation by allogeneic unrelated cells was studied with the MLC microtechniques previously described.13,14 using 10⁶ responding and stimulating cells. The allogeneic stimulating cells either were derived from two different individuals or consisted of a pool of cryopreserved cells from four different individuals.15 Results were expressed in counts/min as the mean of triplicate cultures.
**Lymphocyte response to phytohemagglutinin (PHA), pokeweed mitogen (PWM), and concanavalin A (con A).** Lymphocyte responses to mitogens were assayed by culturing 10⁵ lymphocytes with appropriate dilutions of PHA, con A, and PWM.¹³,⁴ PHA (PHA-P, Difco, Detroit, Mich.) was added at a concentration of 10 µg/well and both PWM (Grand Island Biological, Grand Island, N.Y.) and con A (American Scientific Chemical, Seattle, Wash.) at 5 µg/well. Responses to PHA and PWM were assayed at 72 hr and to con A at 96 hr.

**Humoral immune response in vivo: Isohemagglutinin titers.** IgM anti-A or anti-B titers were determined by standard blood banking hemagglutination tests. IgG isohemagglutinin levels were determined after incubation of sera with 0.1 M 2-mercaptoethanol (2-ME) (v/v) at 37°C for 30 min. Serially diluted sera were incubated with red blood cells at 37°C for 15 min and washed three times; then 1 drop of anti human IgG serum (Coombs' serum) was added to each tube.

**Studies of antigen clearance and antibody response using bacteriophage φX174 (phage).** Phage was grown, harvested, and purified as previously described.¹⁸ Phage was given intravenously in a dose of 5 × 10¹⁰ to 2 × 10¹¹ PFU/kg body weight to give an initial concentration of 1 × 10¹¹ PFU/ml serum. For the study of phage clearance from the bloodstream, serum samples were obtained from 13 patients before injection, 15 min after injection, and then at intervals for 7 days. The number of PFU/ml serum was determined by the agar overlay method of Adams.¹⁷ Serum for determination of phage-neutralizing antibody titers was collected 1, 2, and 4 wk after primary and secondary phage injections. Antibody activity was expressed as the rate of inactivation or K value (Kv).¹⁸ The lower limit of sensitivity is a Kv of 0.01. IgG antibody activity was determined by treating the serum with 2-ME.¹⁹

**Antibody response to keyhole limpet hemocyanin (KLH).** Live keyhole limpets were obtained from Pacific Biomarine Supply, Venice, Calif. They were bled and KLH was prepared according to the method of Campbell et al.²⁰ The technique of immunization consisted of an intradermal injection of 0.1 mg KLH suspended in 0.1 ml normal saline into a site at the forearm using a 25-gauge needle.²¹,²² Serum samples for antibody determination were obtained before and 1, 2, 4, and 8 wk after primary and secondary immunization. Antibodies were determined by an adaptation of the passive hemagglutination technique described for pneumococcal polysaccharide.²³ Briefly, human O+ red blood cells were coated with 0.1 mg/ml KLH, using 1%, chromic chloride as a coupling agent. Sera were inactivated at 56°C for 30 min, and the test was carried out in microtiter plates. IgG antibody activity was determined by using 0.1 M 2-ME.

**Cellular immune response in vivo: Skin reactivity to recall and neoantigens.** The response to skin test antigens was graded as follows: 1+, 5-9 mm induration and/or 10 mm or greater erythema; 2+, 10-20 mm induration; 3+, 21-40 mm induration; 4+, greater than 40 mm induration.²⁴

**Recall antigens.** Reactivity to soluble skin test antigens was determined by injecting 0.1 ml of the following antigens intradermally: Candida (dermatophytin 0, 1:100; Hollister-Stier Laboratories, Spokane, Wash.; mumps (Eli Lilly, Indianapolis, Ind.); intermediate strength purified protein derivative (Parke Davis, Detroit, Mich.); varidase (SK 40 U/SD 10 U/0.1 ml; Lederle, Pearl River, N.Y.); trichophytin (1:10; Hollister-Stier). ²⁵

**Dinitrochlorobenzene (DNCB).** Twenty-five marrow donors were tested with DNCB to confirm the normal response to this substance. Patients were tested at various times after transplant.

**DNCB sensitization.** DNCB 10 mg was dissolved in 0.5 ml acetone and 0.1 ml was applied topically on the skin drop by drop with evaporation of each drop accomplished by blowing air onto the application site. Patients were cautioned not to wash the area for 48 hr. The skin test was observed at 1, 2, and 14 days for response. The responses were graded as follows: 0, no reaction; 1+, erythema only; 2+, erythema and induration; 3+, vesiculation; 4+, ulceration. Any reaction 2+ or greater was considered a positive immune response when observed at 2 or 14 days. Reactions graded 0 were considered negative, and 1+ reactions with erythema were designated as inflammatory responses. Individuals positive after sensitization dose only were not further tested.

**DNCB challenge.** Individuals negative after sensitization were challenged with 0.1 mg DNCB in 0.1 ml of acetone, and the reactions were read at 24 and 48 hr as above. Individuals who failed to show a positive reaction after challenge were subsequently tested in the same sensitization-challenge sequence until positive.

**KLH.** Immunization was carried out as described above.
Statistical Analyses

Data from patients who had GVHD were compared to those from patients without GVHD. Patients were grouped into the following time periods for analysis: 1, 2, 3, 4, 5, 6, 8, 9, 14, 15, 24, 25-36, and greater than 36 mo after transplantation. Patients evaluated more than 15 days after the start of a given month were included in data of the subsequent month. The median value of a test was utilized in the accompanying graphs as representative of the values for the test in a patient group at any given time period. Not all patients were evaluated at every interval because some were occasionally unavailable for study. In all but one instance (antibody to phage, where previously published data were used) data in patients were compared to those of their marrow donors. The Kruskal-Wallace one-way analysis of variance was used to detect differences between groups for all parameters except the skin tests, where \( \chi^2 \) analysis was performed. A correlation analysis was used to evaluate the relationship of bacteriophage titer with respect to time after transplant.

RESULTS

Serum

Immunoglobulins (Fig. 1). IgM levels were in the low normal range in all patients for 3 mo. They remained low for another 6 mo in patients without GVHD and were normal thereafter. In contrast, levels in patients with GVHD were high after the third month (significantly higher than in patients without GVHD, \( p < 0.02 \)), with a return to the normal range at about 1 yr.

IgA levels were in the lower normal range in all patients for about 1 yr and then slowly returned to normal. No difference between patients with and without GVHD was observed.

IgG levels in patients without GVHD were low for about 8 mo and normal thereafter. Patients with GVHD had low levels for 3 mo and significantly higher than normal levels at 8-14 mo (\( p < 0.01 \)); levels returned slowly to normal thereafter. Patients with chronic GVHD had the highest IgG (and also IgM) levels.

Complement (Fig. 2). Levels of both the third and fourth components of
complement were not statistically different from normal in patients with and without GVHD, although C4 was slightly elevated in patients with GVHD 8–24 mo after grafting.

**Lymphocytes**

*Absolute counts* (Fig. 3). Lymphocyte counts in all patients were lower than normal in the first month after transplantation and normal thereafter. The speed of recovery was identical in the two groups of patients.
Lymphocyte surface markers. There were no differences in numbers of E-RFC between patients without and with GVHD (Fig. 4). Both groups of patients showed values in the low normal range for 3 mo and were normal thereafter.

Because the EAC rosette test was performed using either rabbit anti-sheep hemolysin or rabbit anti-SRBC 19S fraction, an analysis of data by each technique was performed. There was no statistical difference between the results by either technique. Therefore the values obtained by each technique were combined for this analysis. Numbers of EAC-RFC in patients both without and with GVHD did not show significant deviations from the normal range throughout the entire postgrafting period (Fig. 4). The slight elevation seen in patients with GVHD at 1 and 2–3 yr was not significant.

Similarly, the numbers of SIg-bearing cells in patients did not significantly deviate from the normal range throughout the postgrafting period (Fig. 5). Patients with GVHD had slightly (but not significantly) higher levels than patients without GVHD between 6 and 15 mo.

Lymphocyte response to irradiated lymphocytes from unrelated individuals (Fig. 6). Lymphocyte responses of patients both without and with GVHD were similar throughout the postgrafting period. They were low for 4–5 mo and then returned to normal. Overall, responses were not statistically different from normal except in patients without GVHD at 1 and 2 mo ($p < 0.03$).

Lymphocyte responses to PWM, con A, and PHA (Fig. 7). Because of the small number of patients studied, responses to PWM and con A must be interpreted with caution. Patients both without and with GVHD had low responses for the first 3–5 mo. Values in patients without GVHD returned to normal thereafter, while values remained low in patients with GVHD [values were significantly lower than normal ($p < 0.001$) only at 9–14 mo]. Low values were
more common in patients with chronic GVHD than in those whose GVHD had subsided.

Findings for PHA were similar to those with con A and PWM: patients without GVHD had normal responses after the fourth month, while responses in patients with GVHD remained significantly lower than normal ($p < 0.001$) for more than 1 yr.

**Humoral Immune Responses in Vivo**

*Isohemagglutinin titers (Fig. 8).* For purposes of comparison, results with anti-A and anti-B titers were combined, and changes in titers over time rather than the actual titers are presented. Fifteen marrow donors were repeatedly tested. Their first titer was considered the baseline and designated “0.” Subsequent determinations in these donors did not deviate from that baseline by
more than two dilutions. Values for the marrow donors are represented by the shaded areas in Fig. 8. The same approach was taken for the marrow recipient, with the admission titer (not shown in Fig. 8) as the baseline.

Patients both without and with GVHD showed similar changes: isohemagglutinin titers without 2-ME were lower (not statistically significant) than normal 2–13 mo after grafting and returned to normal thereafter. After treatment of the sera with 2-ME, isohemagglutinin titers (presumably IgG antibody)
were normal in patients without GVHD, while patients with GVHD showed low titers between 3 and 13 mo.

**Phage clearance and antibody titers.** Phage clearance from the blood after primary phage injection was studied in 13 patients between 1 and 22 mo after marrow transplantation. All 13 cleared the phage by day 4, i.e., in a fashion identical to that seen in healthy individuals.

Figure 9 summarizes the results of primary and secondary immune responses of patients to phage regardless of the time of immunization after transplantation. Patients both without and with GVHD had primary antibody titers significantly lower ($p < 0.0005$) than normal. The decrease in response was slightly more pronounced in patients with GVHD. Both groups of patients showed lower (not or marginally statistically significant) secondary antibody responses than normal. The transition from IgM to IgG production in the secondary response was similar in GVHD and non-GVHD patients but was impaired compared to normal controls.

An attempt was made to investigate the influence of time after transplantation on the magnitude of antibody responses to phage. A correlation analysis was carried out using titers 2 wk after primary and secondary immunizations. Results are shown as a scattergram in Fig. 10. Patients immunized within the first 5 mo of grafting had very low antibody titers. Titers, however, rose steadily as the time after transplantation increased (primary response: $r = 0.514, p < 0.004$; secondary response: $r = 0.64, p = 0.002$). Patients without GVHD were able to achieve higher titers earlier than those with GVHD. However, that the number of patients studied is still too small to draw definitive conclusions.
Antibody response to KLH (Fig. 11). Patients both without and with GVHD showed primary and secondary antibody titers that were significantly lower than those of their donors ($p < 0.003$). This was true for total antibody and for antibody after treatment with 2-ME, presumably IgG. There was no significant difference in antibody responses between patients without and with GVHD.

A separate analysis of the influence of time elapsed after transplantation on the magnitude of the antibody responses did not show a clearcut improvement of the responses with time.

Cellular Immune Response In Vivo—Skin Test Reactivity to Recall and Neoantigens

Data presented in Tables 2-4 are based on the assumption that once a patient had a positive skin test to a given antigen he or she would remain positive as time went on. This assumption is based on the observation that 10 or 11 patients who were repeatedly tested with recall antigens continued to show positive skin tests, while only one was negative, and that on just one occasion.

Recall antigens (Table 2). Throughout the period of observation patients both without and with GVHD showed a lower incidence of positive skin test
reactivity than their marrow donors. In fact, almost none of the patients tested 3 mo after grafting responded to skin test antigens. However, reactivity improved with increasing time after transplantation. Patients without GVHD had a higher incidence of positive skin tests than patients with GVHD. As far as can be determined with the relatively small number of patients studied, patients with mild GVHD (grade I) showed equally poor skin test reactivity as those with moderately severe to severe GVHD (grades II–IV).

**DNCB (Table 3)** Patients who had a negative response to a challenging dose were rechallenged at intervals until they responded positively.

Patients with GVHD tested during the first 36 mo after grafting showed significant decreases in the incidence of positive inflammatory and immune responses, while they were normal thereafter. Skin test reactivity in patients without GVHD returned more rapidly to normal than in those with GVHD. None of the ten patients with chronic GVHD had positive skin tests at any time. Patients with grade-I GVHD appeared to recover skin test reactivity no earlier than those with grades II–IV.

**KLH (Table 4)** Both patients without and with GVHD had a lower incidence of positive skin tests than did marrow donors. There was no difference in reactivity between the two groups.

### Table 2. Skin Test Reactivity to Recall Antigens In Patients After Marrow Transplantation

<table>
<thead>
<tr>
<th>Group</th>
<th>Months After Marrow Transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–8</td>
</tr>
<tr>
<td>No. of Patients Positive/No. of Patients Tested</td>
<td>5/9</td>
</tr>
<tr>
<td>Without GVHD</td>
<td>1/12</td>
</tr>
<tr>
<td>With GVHD</td>
<td></td>
</tr>
</tbody>
</table>

*p values*

<table>
<thead>
<tr>
<th></th>
<th>p values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without versus with GVHD</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td>Without GVHD versus donors</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>With GVHD versus donors</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

*All 25 marrow donors tested responded to at least one of the test antigens.*

*χ² analysis.*
Table 3. Skin Test Reactivity to DNCB in Patients After Marrow Transplantation

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Patients Tested</th>
<th>Inflammatory Response</th>
<th>Immune Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-11</td>
<td>12-36</td>
<td>&gt;36</td>
</tr>
<tr>
<td>Without GVHD</td>
<td>4/8</td>
<td>12/14</td>
<td>14/14</td>
</tr>
<tr>
<td>With GVHD</td>
<td>2/14</td>
<td>7/13</td>
<td>11/11</td>
</tr>
</tbody>
</table>

*p values*
- Without versus with GVHD: >0.05 >0.05 >0.05 <0.03 >0.05 >0.05
- Without GVHD versus donors: <0.01 >0.05 >0.05 <0.01 <0.01 >0.05
- With GVHD versus donors: <0.01 <0.01 >0.05 <0.01 <0.01 >0.05

Of 25 marrow donors tested, 24 had inflammatory and immune responses.

*χ² analysis.

DISCUSSION

One important factor limiting the success of marrow transplantation has been infection, frequently seen during the first 4 mo after transplantation in patients with allogeneic and infrequently seen in those with syngeneic marrow grafts.1,2,5 The nature and clinical courses of these infections resemble those seen in patients with primary immunodeficiency.2b,3a

The aim of the current study was to define the extent and duration of immunologic deficiency in marrow graft patients who survived the period of highest risk of death from GVHD and infection and became long-term survivors. A specific question was whether allogeneic marrow transplantation in man would lead to indefinite impairment of immune function or to its ultimate recovery. In addition, the influence of a single episode of GVHD and of continued chronic GVHD on the immune system was investigated.

The kinetics of recovery of the immune system in lethally irradiated hosts given marrow grafts has already been defined to some extent in rodent and canine systems.31,39 Results in mouse irradiation chimeras varied depending on the kind and dose of antigen used, the nature of the immune response measured, and the strains of animals employed. Most studies were limited to periods of 60-100 days after grafting. Overall, the general immunologic recovery in mice was shown to be absent or impaired for weeks or months after grafting. Ultimately syngeneic chimeras showed normal immunologic function, while allogeneic and xenogeneic chimeras were in general less immunologically active.

Table 4. Skin Test Reactivity to KLH in Patients After Marrow Transplantation

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Individuals Tested</th>
<th>Primary Skin Test</th>
<th>Secondary Skin Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marrow donors</td>
<td>2/29</td>
<td>9/13</td>
<td></td>
</tr>
<tr>
<td>Patients without GVHD</td>
<td>1/14</td>
<td>1/9</td>
<td><em>p &lt; 0.01</em></td>
</tr>
<tr>
<td>Patients with GVHD</td>
<td>0/16</td>
<td>4/13</td>
<td><em>p &lt; 0.05</em></td>
</tr>
</tbody>
</table>

χ² analysis: without versus with GVHD, p > 0.05; without GVHD versus donors, p < 0.01; with GVHD versus donors, p < 0.05.
even as late as 300 days after grafting. Qualitative defects were also noted, such as failure of allogeneic mouse chimeras to convert from 19S to 7S antibody synthesis after multiple challenges with SRBC.\textsuperscript{37,38} It appeared from these studies that allogeneic mouse irradiation chimeras were destined to retain an impairment of immune reactivity.

Fifty canine recipients of allogeneic marrow grafts following TBI were studied for immunologic reactivity between 20 days and 8 yr after grafting by a battery of tests including those used on our patients.\textsuperscript{38} Humoral and cellular immunities were found to be deficient during the first 6-12 mo after transplantation. In contrast to mice, canine irradiation chimeras surviving beyond 1 yr showed an almost complete return of immunologic reactivity to normal, including the conversion from IgM to IgG antibody after repeated immunizations with phage. As a clinical correlate to these studies on immune function, long-term canine chimeras were able to live in an unprotected environment without increased susceptibility to infections or other diseases. The reasons for the differences in immunologic recovery between mouse and dog are currently unknown.

A number of studies of immune reactivity in human recipients of allogeneic marrow grafts have been described. Most were carried out in children with severe combined immunodeficiency disease, and improvement, and in some cases full recovery, of cellular and humoral immunity has been reported.\textsuperscript{40-44} Only very few studies have been reported in patients with acute leukemia or AA. One report describes observations on immunologic reactivity in four patients with hematologic malignancy given marrow from HLA-matched siblings following CY.\textsuperscript{45} They had evidence of graft survival of only 69, 91, 128, and 205 days, and results were similar to ours with regard to normal or near-normal immunoglobulin levels, the ability to make antibody in response to cytomegalovirus and herpes zoster infections, and the failure to develop sensitivity to DNCB. In contrast to our observations there was almost no response of peripheral blood lymphocytes to PHA.

Another study reported the experience with 25 patients with acute leukemia or AA who survived more than 2 mo after transplantation, with a median survival of 4 mo.\textsuperscript{46} At the time of the report only 5 patients were alive, at 4, 4.5, 5.5, 16.5, and 17.5 mo. That study also pointed out the deficiency of immune functions seen shortly after transplantation, but no differences between patients with and without GVHD were seen. This may be related to the short observation times.

Bleyer et al. described the profound effect of GVHD and its treatment with antilymphocyte serum on the immune competence of one patient given an HLA-identical sibling marrow graft for the treatment of AML.\textsuperscript{47} Recovery of skin graft reactivity was delayed to beyond 1 yr, while cellular reactivity in vitro was seen at an earlier time. The patient had low immunoglobulin levels, undetectable isohemagglutinin titers, weak responses to diphtheria and tetanus antigens, and no primary and secondary immune responses to KLH.

Finally, we previously reported initial data on 10 of the current 56 allogeneic long-term survivors and on 3 recipients of syngeneic grafts.\textsuperscript{6}

In contrast to the situation in animals, the pattern of the immune recovery
following allogeneic transplantation in man is expected to be more complex because (1) the ages of donors and recipients varied considerably (2-41 yr), (2) patients had a spectrum of diseases for which they were transplanted, e.g., AA, AML, or ALL, (3) the duration of their diseases and their exposure to treatment by cytotoxic and other drugs varied, and (4) the conditioning regimens for marrow transplantation varied, e.g. CY, TBI, or both. Furthermore, a number of variables encountered after transplantation may influence the recovery of immune function. Some patients develop GVHD, while others do not. Nonspecific immunosuppression is caused by the treatment of GVHD with ATG or corticosteroids. The combination of toxic conditioning regimens, postgrafting immunosuppression, and GVHD produces gut damage leading to a malabsorption syndrome that can cause immunodeficiency, as shown in mice placed on a protein-deficient diet. The number of patients studied as well as the restriction of the current observation to long-term survivors does not permit us to delineate the influence of each of these factors.

Some general conclusions can be made from the present study. First, all patients regardless of whether or not they had GVHD and regardless of their underlying disease had pronounced impairment of all immunologic parameters studied during the first 4 mo after grafting.

Second, differences existed in regard to the tempo and pattern of immunologic reconstitution between patients who never had GVHD and those who had GVHD or were still suffering from GVHD. Responses in vitro to PWM, con A, and PHA and antibody titers to bacteriophage all returned to normal earlier in patients without than in those with GVHD. Surprisingly, IgG levels in patients with GVHD were significantly higher than normal 8-24 mo after grafting. The strikingly elevated IgG levels in the absence of good antibody formation in patients with GVHD may be related to the absence of a cellular mechanism regulating IgG production. The remainder of the immune function studies did not reveal marked differences between patients with and without GVHD. Patients with GVHD showed a tendency toward more frequent and more severe infections than patients without GVHD. The role of GVHD in the more pronounced immune deficiency and higher susceptibility to infection is far from clear despite the number of studies with animals and in vitro. To explain the immune deficiency seen in mice after grafting, Gengozian et al. postulated that “an in vivo antigen antibody reaction persists in these animals and consequently affects their ability to respond normally to other antigens.” The fact that among our patients those with chronic GVHD showed an even more pronounced deficiency of cell-mediated immunity than those who had a transient GVHD would support this view.

Third, most patients ultimately regained near-normal immunologic function and no longer showed an unusually high frequency of infections. However, even in these seemingly healthy patients some apparent random deficiencies of immune function persisted, such as the inability to respond to secondary skin tests with KLH, slightly lower than normal primary and secondary antibody responses to bacteriophage and KLH with slightly less than normal IgG antibody activity upon challenge, and an occasional inability to respond to DNCB skin tests.
Finally, one striking finding was that despite the poor immune reactivity in vivo in the first 6–24 mo after grafting, many parameters of immunity in vitro rapidly returned to the normal range. This suggests that our tests and lymphocyte markers currently used in vitro are not good parameters of the real immunologic status after marrow grafting and shows the necessity for developing new methodology in vitro.

The fact that patients with allogeneic marrow grafts ultimately achieve normal or near-normal immune function makes the use of means of accelerating immune reconstitution and thus shortening the period of risk of serious infections very attractive. Current approaches involve infusion of large numbers of donor lymphocytes in addition to the marrow inoculum and the use of thymosin. Additional approaches will consist of nonspecific stimulation of immunity by levamisole and attempts at transferring immunity from the marrow donor to the recipient by transfer factor. These studies not only might benefit the patient with a marrow transplant but also shed light on unresolved basic questions of the immune system.

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Does graft-versus-host disease influence the tempo of immunologic recovery after allogeneic human marrow transplantation? An observation on 56 long-term survivors

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