Reconstruction of the Immune System After Unrelated or Partially Matched T-Cell–Depleted Bone Marrow Transplantation in Children: Immunophenotypic Analysis and Factors Affecting the Speed of Recovery

By Hoon Kook, Fred Goldman, Doug Padley, Roger Giller, Stephen Rumelhart, Myrl Holida, Nita Lee, Charles Peters, Melanie Comito, Dave Huling, and Michael Trigg

We prospectively studied immune reconstitution in 102 chil-
dren who underwent T-lymphocyte depleted bone marrow
transplants using either closely matched unrelated donors
or partially matched familial donors by assaying total lym-
phocyte counts (TLC), T-cell subsets, B cells, and natural
killer cells. TLC, CD3⁺, and CD4⁺ T-cell counts remained de-
pressed until 2 to 3 years postransplant, whereas CD8⁺ T-
cell counts normalized by 18 months, resulting in an inverted
CD4/CD8 ratio until 12 months postransplant. Although the
percentage of NK cells was elevated early postransplant,
their absolute numbers remained normal. CD20⁺ B cells were
depressed until 12 to 18 months postransplant. Factors af-
fecting immunophenotypic recovery were analyzed by non-
parametric statistics. Younger patients tended to have
higher TLC posttransplant. Higher marrow cell doses were
not associated with hastened immunophenotypic recovery.
Graft-versus-host disease (GVHD) and/or its treatment sig-
ificantly delayed the immune reconstitution of CD3⁺, CD4⁺,
and CD20⁺ cells. The presence of cytomegalovirus was asso-
ciated with increased CD8⁺ counts and a decrease in the
percentages of CD4⁺ and CD20⁺ cells.

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B ONE MARROW transplantation (BMT) has become an
established treatment modality for numerous otherwise
lethal pediatric conditions, including a variety of immuno-
logic, hematologic, metabolic, and oncologic disorders. Lim-
itations to the use of BMT are imposed by the lack of avail-
able histocompatible related donors. To resolve the donor
availability dilemma, three approaches have been taken.
First, transplantation of autologous BM or peripheral blood
stem cells for patients with leukemias and lymphomas have
been used. However, the contamination of the graft by malig-
nant cells, as well as the lack of a beneficial "graft-versus-
malignancy" effect render these patients susceptible to re-
relapse. Moreover, patients having nonmalignant diseases may
not benefit from autologous transplants. The second option
is the use of marrow from closely matched unrelated donors.
This approach has gained more success and acceptance with
the accrual of large numbers of volunteer marrow donors and
with accumulating experience. The third alternative in-
volves the use of histoincompatible or partially matched
family donors. Unfortunately, the high incidence of graft-
versus-host disease (GVHD) in mismatched donors often
necessitates T-cell depletion of the graft and/or the more
aggressive immunosuppression.

Recipients of unrelated donor or HLA-nonidentical trans-
plants appear to have a higher rate of infectious complica-
tions than matched sibling transplant recipients. Inter-
restingly, a variety of infections make up the leading cause
death in our institution, far exceeding those from leukemic
relapse, GVHD, or graft rejection. The infection rate may
be related to the speed of engraftment, the rate of immune
recovery, or the patient’s general resistance to infection.

Immune reconstitution after BMT has been well described
in adults, but only a few reports have been published on
the pediatric population. Moreover, there are no studies
detailing immunologic reconstitution exclusively unrel-
related or partially matched, T-cell–depleted BMT in children
or adults. In this report, we present results of a prospective
study on immunophenotypic recovery of lymphocytes after
T-cell–depleted BMT using either closely matched unrelated
or partially matched familial donors in children. Potential
associated factors were analyzed, including age, sex match
between donor and recipient, marrow cell dose, donor type,
GVHD, and cytomegalovirus (CMV) status. These data have
been used in designing strategies to prevent infections and
accelerate immune recovery.

MATERIALS AND METHODS

Patient population. During 87 months from June 1986 to August
1993, a total of 239 transplants were performed on 209 pediatric
patients at The University of Iowa Hospitals and Clinics (UIHC).
Because this study focused on transplants using unrelated or partially
matched familial donors, HLA-matched sibling transplants (n = 27)
and autologous transplants (n = 9) were not included. To assess
immunologic recovery, patients who survived less than 3 months
(n = 63), received transplants after June 1993 (n = 8), failed to achieve
successful lymphohematopoietic engraftment (ie, early relapse of
malignancy, autologous recovery, or mixed chimerism) (n = 25),
or received multiple transplants (n = 28) were excluded from the
analysis.

A total of 102 patients remained eligible for the study, of which
89% had leukemia. Patient characteristics are listed in Table 1.
Median age was 8.1 years (1.1 to 18.4). Median BM nucleated cell
dose after T-cell depletion was 3.1 × 10⁶/kg of recipient’s body
weight (0.9 to 10.1 × 10⁶/kg). Eighty-eight patients received marrow
from partially matched familial donors (1, 2, or 3 antigen mis-
matched), while 14 had closely matched unrelated donors. Sixty-
one percent of patients showed no evidence of significant GVHD
(Grade 0 or I); 39% developed a Grade

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transplant or the presence of positive systemic CMV culture (eg, urine, microparticle enzyme immunoassay (MEIA) method posttransplant. Twenty-four patients were classified as positive for CMV. CMV IgG titers were not taken into consideration because we routinely administered intravenous immune globulin weekly after transplant. The mononuclear cell fraction was isolated by Ficoll-Hypaque gradient centrifugation. Aliquots of 2 \times 10^8 cells were incubated at 4°C for 30 minutes with the primary antibody, washed once, and then stained with a secondary fluorescin or phycoerythrin-labeled mouse monoclonal antibody (MoAb) and rabbit serum as a source of complement as previously published. In addition to T-lymphocyte depletion of donor marrow, all patients received antilymphocyte globulin (ALG) and prednisone as GVHD prophylaxis. ALG was started 1 day before transplant at a dose of 20 mg/kg and was given every other day until the total white blood cell count reached 1,000/mm^3 or until day +13. Methylprednisolone was given on a daily basis alternating between doses of 1 mg/kg and 2 mg/kg and was usually tapered and discontinued within 6 months of the date of transplant. The presence of GVHD was managed primarily with an increased dose of steroids. Cyclosporine A was reserved for those patients who were unresponsive to steroids.

**Immunofluorescence staining and flow cytometric analysis.** Five milliliters of heparinized peripheral blood was prospectively obtained at 1, 2, 3, 6, 9, 12, 18, 24, 36, 48, 60, and 72 hours after transplantation. The mononuclear cell fraction was isolated by Ficoll-Hypaque gradient centrifugation. Aliquots of 2 \times 10^8 cells were incubated at 4°C for 30 minutes with the primary antibody, washed once, and then stained with a secondary fluorescin or phycoerythrin-labeled goat antimouse antibody. The primary (MoAbs) used in this study were purchased from Becton Dickinson (Mountain View, CA) and included markers of T cells (CD3 (T3/Leu4), CD4 (T4/Leu3), CD8 (T8/Leu2)), B cells (CD19 (B4/Leu12), CD20 (B1/Leu16)), and NK cells (CD16/56 (Leu11c/Leu19)). Fluorescence was expressed using a FACSScan cytofluorometer (Becton Dickinson) at the FACScalculator and the FACSciL. A full blood count was performed on the same day to calculate the absolute number of lymphocytes.

**Controls.** Normal reference values for flow cytometric analysis of lymphocyte populations were assembled from several sources due to a lack of established reference ranges for children. Statistical analysis and plotting. Distributions were asymmetric for most of the lymphocyte subsets. This asymmetry limited the descriptive utility of the arithmetic mean and standard deviation (SD) as measures of central tendency and population dispersion. For this reason, the median \pm standard error (SE) were used as quantitative descriptors of cell population distribution. The percentage and absolute number of peripheral blood mononuclear cells (PBMC) expressing specific surface antigens (CD3, CD4, CD8, CD19, CD20, and CD16/56) were aggregated for each patient over the studied time sequences. Immunophenotypic recovery was defined as the time when the median of each lymphocyte subpopulation surpassed the fifth percentile for the normal population. Patients were subdivided according to characteristics, and the percentage and absolute number of each phenotype were plotted according to the subgroups. The median values of each immunophenotype for each time point were

### Table 1. Patient Eligibility and Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Eligible Patients</th>
<th>No.</th>
</tr>
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<tbody>
<tr>
<td>Total</td>
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<td>102</td>
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<tr>
<td>ALL</td>
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<td>ANLL</td>
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<td>17</td>
</tr>
<tr>
<td>CML (JCML)</td>
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<td>13</td>
</tr>
<tr>
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<td>5</td>
</tr>
<tr>
<td>Wiscott-Aldrich syndrome</td>
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<td>1</td>
</tr>
<tr>
<td>Hurler syndrome</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Metachromatic leukodystrophy</td>
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<td>2</td>
</tr>
<tr>
<td>SAA (Fascon's)</td>
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<td>4</td>
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<tr>
<td>Solid tumors</td>
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<tr>
<td>Rhabdomyosarcoma</td>
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<td>1</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
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</tbody>
</table>

**Age**

- Mean ± SD: 8.6 ± 4.6
- Median, 8.1
- Range, 1.1 to 18.4

**Sex**

- Male: 66
- Female: 36

**Donor**

- Partially matched related: 88
- Closely matched unrelated: 14

**GVHD**

- No GVHD: 62
- Acute GVHD (Grade II or more): 11
- Chronic GVHD (Grade II or more): 13
- Acute + chronic GVHD: 16

**CMV status†**

- Negative: 78
- Positive: 24

* Sex match of donor to recipient.
† CMV positivity was defined by a positive CMV-IgM response posttransplant or the presence of positive systemic CMV culture (eg, urine, blood, other body fluid).
COMMENTS AFTER MARROW TRANSPLANTATION

IMMUNE RECOVERY AFTER MARROW TRANSPLANTATION

Fig 1. Rate and pattern of recovery of lymphocyte populations. Median ± SE of (A) total lymphocyte counts; (B) CD3⁺, (C) CD4⁺, (D) CD8⁺, (E) CD16/CD56⁺, and (F) CD20⁺ cells for 72 months following T-cell-depleted bone marrow transplants. Numbers denote patients analyzed at each point. Normal ranges are shown in shaded areas.

The change in TLCs, T-cell subsets, B cells, and natural killer (NK) cells are shown in Fig 1, with the median ± SE in time sequence up to 72 months posttransplant displayed. The numbers of patients analyzed at each point are indicated on the graphs. These numbers vary, as some patients were analyzed early and subsequently died, whereas other patients have not yet reached the later time points. The normal range for children is shown as shaded areas on the graph.

Mean TLCs fell within the normal range by 24 months posttransplant (Fig 1A). CD3⁺ and CD4⁺ cell numbers recovered slower than CD8⁺ cell numbers, and as a result, the CD4:CD8 ratio remained severely depressed early after transplant, normalizing by 1 year (Fig 2). NK cell number (CD16⁺/CD56⁺) remained within the normal range throughout the posttransplant period (Fig 1E). CD19⁺ and CD20⁺ B-cell numbers normalized by 18 months after transplant.

The percentage of each lymphocyte subpopulation at varying times posttransplant is shown in Fig 3. Percentages of CD3⁺ cells normalized by 18 months after transplant (Fig 3A). The percent of CD8⁺ cells was within the normal range as early as the first month after transplant, while the percent of CD4⁺ cells normalized by 12 months. Interestingly, the percentage of NK cells was highest during the early posttransplant months (making up more than half of the total lymphocytes at 1 month), and eventually normalized by 12 months posttransplant (Fig 3D). Percentages of B cells remained depressed until 18 months after transplant (Fig 3E). The sequential change in percentage of lymphocyte subpopulations is illustrated in pie diagram form (Fig 4). The total sum of these percentages varied due to the possible contribution of null cells and monocytes, which we did not define by MoAbs, and the coexpression of various surface markers on the same cell (eg, CD3 and CD4, CD3 and CD8, CD8 and CD56).

RESULTS

The change in TLCs, T-cell subsets, B cells, and natural killer (NK) cells are shown in Fig 1, with the median ± SE in time sequence up to 72 months posttransplant displayed. The numbers of patients analyzed at each point are indicated on the graphs. These numbers vary, as some patients were analyzed early and subsequently died, whereas other patients have not yet reached the later time points. The normal range for children is shown as shaded areas on the graph.

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In the second part of this study, we analyzed the influence of select patient characteristics on the kinetics of immune reconstitution (Table 2). Patients were subdivided into three age groups and respective subpopulations were compared by the Kruskal-Wallis test. Younger patients (<5 years) tended to have higher TLCs than their older counterparts (>10 years). Except for the TLCs, age did not influence immunophenotypic recovery.

Patients were divided according to administered marrow nucleated cell dose after T-cell depletion (> or <3 x 10⁸ cells/kg of recipient body weight). The number of NK cells was higher in patients who received more than 3 x 10⁶ cells/kg for the first 3 months, and this was statistically significant at 2 months (P = .019) (Table 2). However, both groups were within normal ranges throughout the posttransplant period. Total lymphocyte number, numbers of CD3⁺, CD4⁺, and CD20⁺ cells were significantly higher in the group given a larger marrow cell dose for the first 1 to 2 months posttransplant (Table 2). However, higher marrow doses were not associated with accelerated immunophenotypic recovery at
A B C D E

Fig 3. Rate and pattern of percentage recovery of lymphocyte subpopulations. Median ± SE of (A) CD3+, (B) CD4+, (C) CD8+, (D) CD16+/CD56+, and (E) CD20+ cells for 72 months following T-cell-depleted bone marrow transplants. Normal ranges are shown in shaded areas.

DISCUSSION

Severe, life-threatening infection is a serious obstacle to a successful BMT outcome. The high risk of infection may be a direct consequence of a naive or dysfunctional immune system in the early posttransplant period. All components of the immune system including mucous membrane defense system, phagocytic and accessory cell functions, lymphocyte cytotoxic function, T-cell–mediated immunity, and B-cell function may be affected. Immunologic dysfunction is also influenced by several other factors, including the underlying disease for which the BMT was performed, the degree of HLA compatibility, the time to engraftment, the presence of GVHD, the method of prophylaxis against GVHD, the treatment of GVHD, and CMV status (presence of infection or immunity) before BMT.6,15 Intensive efforts are often directed at protecting patients from infections during this period of profound immune deficiency.

Immune reconstitution after BMT has been widely studied in adults.7,8 Recovery of humoral and cellular immunity may take 1 year or longer depending on whether the recipients develop GVHD. Both acute and chronic GVHD slow the rate of immune reconstitution.16 It has been proposed that GVHD induces thymic epithelial damage with a resultant specific CD4+ cell functional defect.17 In addition, immunosuppressive drugs used to prevent or treat GVHD including antilymphocyte globulin, prednisolone, cyclosporine, and methotrexate impede immune recovery.18 HLA disparity between donor and recipient may further delay immune recovery, resulting in a higher incidence of later time points. Donor types, whether closely matched unrelated donor or partially matched family donor, and sex matches from donor to recipient did not influence immunophenotypic recovery.

Chronic GVHD or combined acute plus chronic GVHD significantly retarded the normalization of CD3+, CD4+, and CD20+ cell numbers and CD4+, CD20+, and NK cell percentages (Table 2). Patients without GVHD had higher CD20+ cell counts than patients with chronic GVHD or combined acute plus chronic GVHD patients (Fig 5). These differences were statistically significant between 6 and 18 months posttransplant.

The influence of CMV was examined in our patient population. Total lymphocyte number, numbers and percentages of CD3+, CD8+, and NK cells were statistically higher in CMV+ patients (Table 2). In contrast, the percentage of CD4+ cells with resultant inverted CD4:CD8 ratio and the percentage of CD20+ cells tended to be lower with CMV infection. The differences were significant from 6 to 9 months posttransplant. Moreover, patients positive for CMV showed a significantly different immunophenotypic recovery in several parameters (Table 3).

CMV+ patients had a delayed recovery of CD4:CD8 ratio as well as CD4+ and CD20+ cell percentages. CMV+ patients attained a normal CD4:CD8 ratio by 12 months compared with 48 months for CMV+ patients (Fig 6A).

Fig 4. Proportion of T cells and NK cell population after transplant.
### Table 2. Immunophenotypic Reconstitution Following T-Cell-Depleted BMT According to Patient Characteristics:

<table>
<thead>
<tr>
<th>Cell Dose (M-W)</th>
<th>GVHD (K-W)</th>
<th>CMV (K-W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3×10⁸ &gt; 3×10⁸</td>
<td>No/Acute/Chronic/Combined</td>
<td>Positive/Negative</td>
</tr>
</tbody>
</table>

| Time of Significant Difference | Total lymphocytes | CD3 No. | CD3 percentage | CD4 No. | CD4 percentage | CD8 No. | CD8 percentage | CD4:CD8 ratio | NK No. | NK percentage | CD20 No. | CD20 percentage |
|-------------------------------|-------------------|---------|----------------|---------|----------------|---------|----------------|---------------|--------|--------------|---------|----------------|----------------|
| Months                        | P                 |         |                |         |                |         |                |               |        |              |         |                |
| 2                             | .044              |         |                |         |                |         |                |               |        |              |         |                |
| 24                            | .036              |         |                |         |                |         |                |               |        |              |         |                |
| 1                             | .0067             |         |                |         |                |         |                |               |        |              |         |                |
| 24                            | .032              |         |                |         |                |         |                |               |        |              |         |                |
| 6                             | .0002             |         |                |         |                |         |                |               |        |              |         |                |
| 9                             | .01               |         |                |         |                |         |                |               |        |              |         |                |
| 2                             | .021              |         |                |         |                |         |                |               |        |              |         |                |
| 12                            | .045              |         |                |         |                |         |                |               |        |              |         |                |
| 18                            | .0084             |         |                |         |                |         |                |               |        |              |         |                |
| 9                             | .0371             |         |                |         |                |         |                |               |        |              |         |                |
| 18                            | .0401             |         |                |         |                |         |                |               |        |              |         |                |
| 6                             | .001             |         |                |         |                |         |                |               |        |              |         |                |
| 3                             | .017              |         |                |         |                |         |                |               |        |              |         |                |
| 6                             | .004              |         |                |         |                |         |                |               |        |              |         |                |
| 9                             | .011              |         |                |         |                |         |                |               |        |              |         |                |
| 12                            | .0004             |         |                |         |                |         |                |               |        |              |         |                |
| 18                            | .0016             |         |                |         |                |         |                |               |        |              |         |                |
| 2                             | .017              |         |                |         |                |         |                |               |        |              |         |                |
| 3                             | .0391             |         |                |         |                |         |                |               |        |              |         |                |
| 6                             | .00331            |         |                |         |                |         |                |               |        |              |         |                |
| 9                             | .00081            |         |                |         |                |         |                |               |        |              |         |                |
| 12                            | .00081            |         |                |         |                |         |                |               |        |              |         |                |
| 18                            | .00141            |         |                |         |                |         |                |               |        |              |         |                |
| 6                             | .005              |         |                |         |                |         |                |               |        |              |         |                |
| 9                             | .030              |         |                |         |                |         |                |               |        |              |         |                |
| 18                            | .0058             |         |                |         |                |         |                |               |        |              |         |                |
| 1                             | .047              |         |                |         |                |         |                |               |        |              |         |                |
| 12                            | .024              |         |                |         |                |         |                |               |        |              |         |                |
| 18                            | .0079             |         |                |         |                |         |                |               |        |              |         |                |
| 2                             | .0079             |         |                |         |                |         |                |               |        |              |         |                |
| 9                             | .00033            |         |                |         |                |         |                |               |        |              |         |                |
| 12                            | .00086            |         |                |         |                |         |                |               |        |              |         |                |
| 24                            | .045              |         |                |         |                |         |                |               |        |              |         |                |
| 3                             | .0019             |         |                |         |                |         |                |               |        |              |         |                |
| 2                             | .0044             |         |                |         |                |         |                |               |        |              |         |                |
| 12                            | .0044             |         |                |         |                |         |                |               |        |              |         |                |
| 18                            | .0009             |         |                |         |                |         |                |               |        |              |         |                |
| 2                             | .0009             |         |                |         |                |         |                |               |        |              |         |                |
| 6                             | .0036             |         |                |         |                |         |                |               |        |              |         |                |
| 9                             | .0013             |         |                |         |                |         |                |               |        |              |         |                |
| 12                            | .028              |         |                |         |                |         |                |               |        |              |         |                |

Abbreviations: GVHD, Graft-versus-host disease; CMV, cytomegalovirus; K-W, Kruskal-Wallis; M-W, Mann-Whitney.

* Patients with GVHD had lower value than patients without GVHD, except for NK number, which showed lower value that was closer to normal for patients without GVHD.

† Patients with CMV positivity showed lower level for each immunophenotypic cells than CMV negative patients (see text). Donor types whether closely matched unrelated or mismatched familial donor and sex matches between donor and recipients did not affect the numbers and percentages of lymphocyte population.

In this study, immunophenotypic recovery was prospectively analyzed in children receiving T-cell-depleted BMTs. In our patient population, TLCs returned to normal by 24 months posttransplant. This contrasts with other reports where TLC returned to normal by 3 months in adults undergoing matched sibling transplants and by 9 months in children receiving T-cell-replete or T-cell-depleted BMT. In mismatched transplants, T-cell depletion of the marrow is often required to prevent severe GVHD. The effect of T-cell depletion of donor marrow on immune recovery is still controversial. Some reports showed no differences between T-cell replete and T-cell--depleted transplants, while others found slower recovery in patients receiving T-cell-depleted marrow. It is suggested that T-cell depletion removes "immunocompetent" donor-derived cells and this slows the rate of engraftment of myeloid and/or lymphoid cells. Infections in mismatched transplants, T-cell depletion of the marrow is often required to prevent severe GVHD. The effect of T-cell depletion of donor marrow on immune recovery is still controversial. Some reports showed no differences between T-cell replete and T-cell--depleted transplants, while others found slower recovery in patients receiving T-cell-depleted marrow. It is suggested that T-cell depletion removes "immunocompetent" donor-derived cells and this slows the rate of engraftment of myeloid and/or lymphoid cells.
patients are the higher degree of mismatch or the intensity and chronic GVHD. By Kruskal-Wallis, no GVHD; (●) acute GVHD; (△) chronic GVHD; (□) combined acute and chronic GVHD. By Kruskal-Wallis, @, P < .001 for □ v △; #, P = .004 for □ v △ and P = .004 for □ v △; $, P = .001 for □ v △ and P = .001 for □ v △; %, P = .028 for □ v △; +, z = 2.53 for □ v △.

The life span of NK cells is not well characterized; ranging from a few days to several months. Their early appearance following BMT raises questions about the origin of NK cells. Although most reports indicate donor origin, their resistance to radiation could allow recipient NK cells to exist post-BMT. Fluorescent in situ hybridization with Y-chromosome probe after positive selection of NK cells could be helpful in determining NK cell origin in sex mismatched transplants. We defined NK cells as CD16+/56+ cells, although this criterion might include a small proportion (<5%) of mature T cells (CD3+, CD16+/56+). In this study, the absolute number of NK cells remained normal throughout the posttransplant period, whereas the percentage of NK cells peaked early posttransplant, returning to normal by 12 months.

For B-cell enumeration, MoAbs to CD19 were preferred to CD20, as CD19 is expressed on developing B cells, whereas CD20 can be present on a subpopulation of T cells. In our study, children were found to be B-cell deficient for a long period of time. The delay in B-cell reconstitution, similar to the delay in T-cell engraftment, may be related to our use of antilymphocyte globulin (which would be expected to have reactivity against B and T cells) and prolonged immune suppression.

Patient characteristics were analyzed to correlate their impact on the immunophenotypic recovery of lymphocytes. Younger patients seemed to have significantly higher lymphocyte counts after transplant in this study. Others have shown that younger donor age and younger recipient age, to a lesser degree, were associated with higher lymphocyte numbers and faster rate of recovery. This trend might be expected considering the relative naivety of the immune system of CD2− or CD3+ cells within 6 months postgrafting in adults, or at most 1 year in children. Possible explanations for the observed delayed lymphoid reconstitution in our patients are the higher degree of mismatch or the intensity of our conditioning regimen that could adversely affect the marrow stroma needed to support lymphopoiesis. Perhaps more likely is our use of antilymphocyte globulin (which would be expected considering the relative naivety of the immune system) and prolonged use of steroids as prophylaxis of GVHD in addition to T-cell depletion of BM.

Most studies indicate that the helper/inducer CD4+ T cells are reduced both in relative proportions and in absolute numbers during the early posttransplant period. T-cell–depleted patients showed more exaggerated depression than the T-cell–replete group, and the recovery of absolute numbers of CD4+ cells was greatly delayed. On the other hand, the recovery of CD8+ cells is controversial. The number and proportion of CD8+ cells quickly normalized and remained elevated long-term posttransplant in recipients of unmanipulated marrow, or in those receiving T-cell–depleted transplants. However, this is not found in all recipients of T-cell–depleted BMTs, suggesting that mature T cells in the donor inoculum might contribute to this CD8+ cell elevation. The increase of CD8+ cells in the previously published studies could be ascribed to the inclusion of dimly fluorescent CD8+ cells that coexpress NK markers. In our report, the absolute number of CD8+ cells recovered slowly, whereas the percentage remained well within normal ranges throughout the posttransplant period. Moreover, an excess of CD8+ cells was not observed during the immediate posttransplant period.

NK cells are large granular lymphocytes capable of mediating antibody-dependent cell-mediated cytotoxicity and may provide antiviral activity. These cells may play a regulatory role in lymphohematopoiesis. Reports in adults have demonstrated an increased number of NK cells in the first month after transplant, constituting a considerable proportion of the circulating mononuclear cells. Even in T-cell–depleted grafts, NK cells appear early and may be responsible for lymphokine production in BMT recipients.

Table 3. Influence of CMV Status on the Immunophenotypic Recovery (mo. to attain normal value)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total Patients</th>
<th>CMV- Patients</th>
<th>CMV+ Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte</td>
<td>24 mo</td>
<td>24 mo</td>
<td>36 mo</td>
</tr>
<tr>
<td>CD3 No.</td>
<td>36 mo</td>
<td>36 mo</td>
<td>18 mo</td>
</tr>
<tr>
<td>CD3 Percentage</td>
<td>18 mo</td>
<td>18 mo</td>
<td>12 mo</td>
</tr>
<tr>
<td>CD4 No.</td>
<td>36 mo</td>
<td>36 mo</td>
<td>36 mo</td>
</tr>
<tr>
<td>CD4 Percentage</td>
<td>12 mo</td>
<td>12 mo</td>
<td>24 mo9</td>
</tr>
<tr>
<td>CD8 No.</td>
<td>18 mo</td>
<td>24 mo</td>
<td>6 mo</td>
</tr>
<tr>
<td>CD8 Percentage</td>
<td>1 mo</td>
<td>2 mo</td>
<td>1 mo</td>
</tr>
<tr>
<td>CD4:CD8 ratio</td>
<td>12 mo</td>
<td>12 mo</td>
<td>NR1</td>
</tr>
<tr>
<td>NK No.</td>
<td>1 mo</td>
<td>1 mo</td>
<td>1 mo</td>
</tr>
<tr>
<td>NK Percentage</td>
<td>12 mo</td>
<td>12 mo</td>
<td>24 mo1</td>
</tr>
<tr>
<td>CD20 No.</td>
<td>12 mo</td>
<td>12 mo</td>
<td>36 mo</td>
</tr>
<tr>
<td>CD20 Percentage</td>
<td>18 mo</td>
<td>18 mo</td>
<td>24 mo</td>
</tr>
</tbody>
</table>

Abbreviation: NR, not reached to normal by 48 months posttransplant.

* P < .05.
† P < .01 significant delay between CMV+ and CMV− groups.
IMMUNE RECOVERY AFTER MARROW TRANSPLANTATION

Fig 6. (A) CD4:CD8 ratio in relation to CMV status (B) NK cell number according to CMV status. (■), no CMV disease; (●), CMV positivity. By Mann-Whitney, *P < .05; **P < .01; ***P < .001.

tem of a younger person and the higher numbers of lymphocytes in normal children.14

Patients who received a nucleated cell bone marrow dose of more than 3 × 10^9/kg of recipient body weight tended to have significantly higher numbers of total lymphocytes, CD3+, CD4+, CD20+, and NK cells for the first 1 to 2 months posttransplant (Table 2). Whether this elevation was attributable to the infusion of phenotypically mature cells with the marrow graft despite T-cell depletion or from faster immunohematopoietic recovery is not clear. Giving higher cell doses might be beneficial, as most fatal infections occur during the first 3 months posttransplant. However, it is not clear whether these cells are functional, and we were unable to see a survival advantage in those receiving higher marrow doses. Furthermore, when later time points were examined, higher marrow doses were not associated with accelerated immune recovery.

It is well known that both GVHD per se and accompanying immunosuppressive drugs delay the recovery of the immune system.15,16 GVHD selects the lymphoid system as a target organ in addition to the skin, liver, and gut. Lymphoid hypocellularity and atrophy are characteristic histologic hallmarks of moderate or severe GVHD. Thymic epithelial injury has been demonstrated with resultant specific CD4+ cell functional defects.17 Moreover, prednisone and cyclosporine A block T-cell effector function and interleukin-2 (IL-2) production. ALG is likely cytotoxic to both T and B cells.17 In studies on patients with chronic GVHD, the proportion of CD8+ cells in the peripheral blood was increased, while the proportion of CD4+ cells was decreased, resulting in a persistently inverted CD4:CD8 ratio.18,19 The role of CD8+ cells in the pathogenesis of GVHD has been suggested by a temporal association and histopathologic studies.20 Some investigators suggest that NK cells can also function as effectors of GVHD.21 From our data, however, we were unable to correlate the number of cytotoxic/suppressor cells or NK cells with the occurrence of GVHD. This may be related to our early aggressive treatment of GVHD. Consistent with other reports, the presence of chronic GVHD (or combined acute and chronic GVHD) significantly retarded the immune reconstitution of mature T cells, helper/inducer T cells, and mature B cells for the first 2 years post-BMT.7,10,22

Several studies have demonstrated that CMV infection moderates the tempo of immunologic recovery post-BMT.8,23 However, because CMV infection was strongly associated with the occurrence of acute GVHD,15,24 the effect of CMV on immunologic maturation must be interpreted with caution. Paulin et al25 reported that patients with CMV infection (but without chronic GVHD) had a more prolonged period of lymphocyte dysfunction. Other studies have found CMV infection can upregulate the number of peripheral CD8+ T cells.26-30 In our study, patients with CMV had a persistently inverted CD4:CD8 ratio, in accordance with other reports.31,32 Moreover, a significantly higher number of total lymphocytes as well as CD3+, CD8+, and CD16+/56+ cell subpopulations were noted in patients with CMV. These increases may simply reflect clonal expansion in response to CMV rather than generalized acceleration of immune recovery. The differences in respective lymphocyte subpopulations in CMV+ patients was most profound from 6 months to 9 months post-BMT, a time interval overlapping with the peak incidence of CMV.15

Although the degree of donor/recipient histocompatibility was suspected to be a predictive factor for immune reconstitution,15 our study was unable to detect differences between partially matched familial donors and closely matched unrelated donors. This might be attributable to the small numbers of patients in the unrelated donor group or our immunosuppressive regimen. Larger studies will be needed to more definitively address the issue of whether donor type is associated with the rate of immune reconstitution.

In this study, we have better defined the kinetics of immune reconstruction following T-cell–depleted BMT in children. Clearly, children receiving T-lymphocyte depleted...
marrow from mismatched familial donors or closely matched unrelated donors remain lymphopenic for many months post-BMT and may benefit from a variety of clinical maneuvers to protect them from infection. Further studies will examine the recovery of immune function and correlate this, as well as the immunophenotype, with various clinical conditions and treatments. These studies may guide clinicians on the duration of prophylactic antimicrobial measures and on modifications of behavior and lifestyle post-BMT.

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REFERENCES

33. Atkinson K: T cell subpopulations defined by monoclonal antibodies after HLA-identical sibling marrow transplantation. II. Activated and functional subsets of the helper-inducer and the cytotoxic-suppressor subpopulations defined by two color fluorescence flow cytometry. Bone Marrow Transplant 1:121, 1986
Reconstruction of the immune system after unrelated or partially matched T-cell-depleted bone marrow transplantation in children: immunophenotypic analysis and factors affecting the speed of recovery

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