Immunologic Resolution of Human Chronic Graft-versus-Host Disease

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ABSTRACT

To determine the role of regulatory T lymphocytes (Tregs) in the pathogenesis of human chronic graft-versus-host disease (GVHD) and its clinical resolution, we evaluated long-term recipients of pediatric allogeneic hematopoietic stem cell transplantation (HSCT). Seventy-one recipients were evaluated, 30 of whom had a history of chronic GVHD, including 16 with active chronic GVHD and 14 with resolved chronic GVHD. There were no significant clinical differences and no differences in the frequency of Tregs (CD4+ CD127− CD25+) between the recipients with active chronic GVHD and those with resolved chronic GVHD. Using the Miyara/Sakaguchi classification scheme to identify functional Tregs, a decreased frequency of functional resting Tregs (rTregs) was identified in recipients with active chronic GVHD (P = .009 compared with normal donors; P = .001 compared with HSCT recipients without history of chronic GVHD; P = .005 compared with recipients with resolved chronic GVHD). The frequency and number of recent thymic emigrants in rTregs were normal in recipients with resolved chronic GVHD, but persistently decreased in recipients with active chronic GVHD. These results support the hypothesis that the reestablishment of normal numbers of functional rTregs is required for the clinical resolution of chronic GVHD.

INTRODUCTION

Given the clinical similarity of chronic graft-versus-host disease (GVHD) to systemic sclerosis and other autoimmune diseases in humans, the idea that the pathogenesis of chronic GVHD differs from that of acute GVHD has been debated for many years [1-3]. It is clear that donor-derived T lymphocytes with specificity for recipient-restricted histocompatibility antigens and the associated cytokines are responsible for the development of acute GVHD; however, the pathogenesis of chronic GVHD is less clear, with recent research focusing on the role of regulatory T lymphocytes (Tregs).

Clinical research into chronic GVHD has been confounded by ambiguities in the definition of human Tregs. In most previous studies, Tregs were immunophenotypically identified by phenotype: CD4+, CD127−, CD25+ (Figure 1A); however, in contrast to mice, in which all functional Tregs express FoxP3, not all human Tregs express FoxP3, and not all FoxP3-expressing human Tregs are functional [4-6]. Miyara et al. [7] developed a classification of human Tregs that permits the immunophenotypic identification and quantitation of functional human Tregs. Two functional Treg subpopulations are identified: resting Tregs (rTregs), representing 20% of the total Tregs in normal individuals and their activated progeny, activated Tregs (aTregs), which represent 5% (Figure 1A). Thus, only 25% of Tregs (CD4+, CD127−, CD25+) have a regulatory function in humans.

We have used the Miyara/Sakaguchi classification of functional human Tregs to quantify the frequency and number of rTregs and aTregs in the long-term pediatric recipients of hematopoietic stem cell transplantation (HSCT). The purpose of the present study was to examine the role of functional Tregs in the development of human chronic GVHD and the clinical resolution of chronic GVHD. Many HSCT recipients with chronic GVHD experience clinical resolution of chronic GVHD after first-line (50% clinical resolution rate) or second-line (15% clinical resolution rate) immunosuppressive therapy, with the ultimate discontinuation of immunosuppression [8,9]. Although the clinical factors that increase the risk of developing chronic GVHD have been identified (eg, previous acute GVHD, older recipient age, use of mobilized peripheral blood stem cells, total body...
irradiation [TBI]-containing conditioning regimen, non-histocompatible donors), the biological basis for the clinical resolution of chronic GVHD has not been investigated.

Our study is the first to demonstrate that HSCT recipients with active chronic GVHD have markedly lower than normal frequency and number of functional rTregs, whereas HSCT recipients with clinical resolution of chronic GVHD have normal values. To better understand the basis for the normalization of rTregs in the recipients with resolved chronic GVHD, we investigated the contribution of the thymus to the rTregs in recipients with active and resolved chronic GVHD by measuring the frequency of recent thymic emigrants (RTEs) within the rTreg subpopulation. Whereas the recipients with active chronic GVHD exhibited sustained decreases in the frequency and numbers of RTEs within their rTregs, those with resolved chronic GVHD demonstrated an initial increase in the frequency of RTEs within their rTreg subpopulation, which normalized over time. Overall, our results suggest that the immunologic resolution of human chronic GVHD is related to the reestablishment of normal immune regulation secondary to the improved thymic production of functional Tregs.

**PATIENTS AND METHODS**

**Patients**

This nonselected cross-sectional evaluation of allogeneic HSCT recipients was conducted between March 2011 and June 2013. All HSCT recipients who were more than 1 year post-transplantation and whose parent/guardian signed an informed consent document approved by the Children’s
BM indicates bone marrow; CB, cord blood; PBSC, peripheral blood stem cells; ATG, anti-thymocyte globulin.

Table 1
Characteristics of the HSCT Recipient Groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 37)</th>
<th>Group 2 (n = 4)</th>
<th>Group 3 (n = 16)</th>
<th>Group 4 (n = 14)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of acute GVHD (grade II-IV)</td>
<td>No</td>
<td>Yes</td>
<td>Yes/no</td>
<td>Yes/no</td>
<td></td>
</tr>
<tr>
<td>History of chronic GVHD</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Chronic GVHD status</td>
<td>–</td>
<td>–</td>
<td>Active</td>
<td>Resolved</td>
<td></td>
</tr>
</tbody>
</table>

Hospital Los Angeles Institutional Review Board were entered into the study. After the completion of recipient enrolment, the acute and chronic GVHD status of each recipient was rescoped by an investigator (R.P.) using the Glucksberg criteria to score acute GVHD and the National Institutes of Health consensus criteria to grade chronic GVHD [10,11]. The investigator was blinded to the results of immunologic testing at the time of the rescoping. Ultimately, only recipients who underwent transplantation at Children’s Hospital Los Angeles, who had received only 1 transplant, and who had received myeloablative conditioning (except for patients with severe aplastic anemia) were included. Thus, patients with severe combined immune deficiency, who did not receive myeloablative conditioning, were excluded.

Each patient was assigned to 1 of 4 groups: group 1, grade 0-1 acute GVHD and no history of clinical chronic GVHD; group 2, grade II-IV acute GVHD and no history of clinical chronic GVHD; group 3, active clinical chronic GVHD or a history of chronic GVHD but no clinical chronic GVHD and receiving 2 or more systemic immunosuppressive drugs; or group 4, history of chronic GVHD but no signs of clinical chronic GVHD and off of all immunosuppressant therapy or receiving minimal doses of a single systemic immunosuppressive drug (Table 1). The maximal clinical severity of chronic GVHD was scored. If more than 1 immunologic evaluation was performed, the results from the first complete evaluation were used.

Normal pediatric bone marrow transplant donors whose parent/guardian provided signed informed consent served as controls.

Immunophenotypic Analyses

Human peripheral blood was collected in heparin, and mononuclear cells were isolated on Ficoll-Hypaque gradients and cryopreserved. Thawed mononuclear cells were stained with anti-CD4 (FITC, BD Pharmingen, San Diego, CA), anti-CD127 (Alexa Fluor 647; BD Pharmingen), anti-CD25 (PE-Cy7; BD Pharmingen), CD45RA (PE-Cy5; BD Pharmingen), and anti-FoxP3 (PE, clone 236A/E7; e-Bioscience; San Diego, CA) antibodies. Intracellular antigen detection was performed on cells fixed and permeabilized with Cytofix/Cytoperm (e-Bioscience). CD4 T lymphocytes, conventional T lymphocytes (Tc0; CD4, CD127, CD25, CD45RA), and rTregs (CD4, CD127, CD25, CD45RA, FoxP3+) were quantified.

Functional Treg subpopulations—resting Tregs (rTregs; CD4+, CD127−, CD25−, CD45RA+, FoxP3+) and aTregs (CD4+, CD127−, CD25+, CD45RA−, FoxP3−+)—were identified using a sequential gating strategy: FCS/CD4→CD127/CD25→CD45RA/FoxP3 (Figure 1A) [7]. rTregs (Figure 1A, III, P12) were defined as CD45RA− cells with FoxP3 staining >99.9% of the isotype control. aTregs (Figure 1A, III, P9) were defined as CD45RA− cells with FoxP3 staining >99.9% of the FoxP3 staining of the rTregs. Treg subpopulations were measured as a percentage of CD4 T lymphocytes or total Tregs and as lymphocytes/µL. Functional analyses of the Treg lymphocyte subpopulations were not performed because fixation was required for the FoxP3 staining. RTEs were identified as CD4−, CD45RA−, CD3− T lymphocytes using anti-CD3 antibody (V450; e-Bioscience) (Figure 1B). RTEs were measured as percentage of the CD4 T lymphocyte subpopulations or as...
lymphocytes/μL. Data were acquired on a FACSCanto II flow cytometry system (BD Biosciences, San Jose, CA) and analyzed with BD FACSDiva software.

Statistical analyses of the FACS results were performed using the Mann-Whitney U test. Prism version 6 (GraphPad Systems, San Diego, CA) was used for statistical analyses (Fisher’s exact, chi-square, ANOVA, Mann-Whitney, and linear regression analyses) and graphic generation.

RESULTS

Patient Characteristics

Eighty-nine pediatric HSCT recipients were initially entered into the study, which resulted in 71 evaluable subjects. The 18 recipients who were not included included 5 who did not undergo transplantation at Children’s Hospital Los Angeles, 3 who had received 2 transplants, 5 who had severe combined immune deficiency and did not receive myeloablative conditioning, and 5 who did not have adequate immune evaluation data.

The shortest time to immune evaluation was 12 months and the longest was 208 months, with a mean of 83 months. Of the 71 evaluable recipients, 37 were in group 1 (no history of either acute GVHD grade II-IV or chronic GVHD), 4 were in group 2 (only acute GVHD grade II-IV, with no history of chronic GVHD), 16 were in group 3 (active chronic GVHD), and 14 were in group 4 (resolved chronic GVHD) (Table 1). No significant differences among the 4 groups were found. The recipient sex, donor identity (Table 1). No significant differences among the 4 groups were found.

The groups differed only in the containing regimens (Table 2). The groups differed only in the stem cell source, were not signifi cantly associated with the development of clinical chronic GVHD. Of the 14 recipients with resolved chronic GVHD, 10 were off of all immunosuppressive therapy for a mean duration of 29 months, 3 were resolved chronic GVHD, 16 were in group 3 (active chronic GVHD), and 14 were in group 4 (resolved chronic GVHD) (Table 1). No significant differences among the 4 groups were found. In the present study, we found normal frequencies and numbers of rTregs in pediatric HSCT recipients with resolved chronic GVHD (group 4), but decreased frequencies and numbers in recipients with active chronic GVHD (group 3). The increased thymic production of rTregs may be a possible mechanism for the normalization of rTregs in the HSCT recipients with resolved chronic GVHD. To directly determine the status of thymic function in the HSCT recipients, we evaluated the frequencies of RTEs (CD4+, CD45RA+, CD25+, FoxP3+; P12) and their progeny, aTregs (CD4+, CD127+, CD25+, CD45RA+, FoxP3+++; P9) [7]. Regulatory function is associated only with the rTreg and aTreg subpopulations, and not with the other FoxP3-expressing subpopulation of the FoxP3-negative subpopulation (Figure 1A).

The HSCT recipients were assessed for frequency of rTregs as percentages of both CD4 T lymphocytes and Tregs (Figure 2B). The Miyara classification scheme permits the immunophenotypic identification of the functional Treg subpopulations, including rTregs (CD4+, CD127+, CD25+, CD45RA+, FoxP3+; P12) and their progeny, aTregs (CD4+, CD127+, CD25+, CD45RA+, FoxP3+++; P9) [7]. Regulatory function is associated only with the rTreg and aTreg subpopulations, and not with the other FoxP3-expressing subpopulation of the FoxP3-negative subpopulation (Figure 1A).

The frequency of rTregs in group 3 recipients was decreased, whereas the frequency in group 1 and group 4 recipients was similar to that in normal pediatric donors. The frequency of rTregs in the group 3 recipients was lower than that of normal donors (P = .009), group 1 recipients (P = .001), and group 4 recipients (P = .005). Analysis of the absolute numbers of rTregs (rTregs/μL) demonstrated almost identical results (Figure 3A). When only the group 4 recipients who were off of all immunosuppression were analyzed, the frequency of rTregs in the group 3 recipients still differed from that of the group 4 recipients (P = .01).

Assessment of T Lymphocytes

We analyzed the pediatric HSCT recipients for the frequency of CD4 T lymphocytes, Tcons, and Tregs (CD4+, CD127+, CD25+). No differences among the GVHD groups were identified (Figure 2A). In addition, there were no differences in the absolute number of CD4 T lymphocytes

### Table 3
Clinical Characteristics of Recipients with Active (Group 3) or Resolved (Group 4) Chronic GVHD

<table>
<thead>
<tr>
<th></th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Recipients</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Organ Involvement</td>
<td>Skin</td>
<td>Gl</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Time to Evaluation, mo</td>
<td>73.6</td>
<td>86.7</td>
</tr>
</tbody>
</table>

Gl indicates gastrointestinal tract.

### Table 4
Immunosuppression of Recipients with Active (Group 3) or Resolved (Group 4) Chronic GVHD

<table>
<thead>
<tr>
<th>Immunosuppression</th>
<th>T/S/C</th>
<th>Steroids</th>
<th>Daclizumab</th>
<th>Azathioprine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 3, previous therapy</td>
<td>13</td>
<td>9</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Group 4, therapy at evaluation</td>
<td>12</td>
<td>8</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>Group 4, previous therapy</td>
<td>13</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

T/S/C indicates tacrolimus/sirolimus/cyclosporine.

Thymic Function

In the present study, we found normal frequencies and numbers of rTregs in pediatric HSCT recipients with resolved chronic GVHD (group 4), but decreased frequencies and numbers in recipients with active chronic GVHD (group 3). The increased thymic production of rTregs may be a possible mechanism for the normalization of rTregs in the HSCT recipients with resolved chronic GVHD. To directly determine the status of thymic function in the HSCT recipients, we evaluated the frequencies of RTEs (CD4+, CD45RA+, CD31+) in total CD4 T lymphocytes, Tcons, and Tregs. We found an increased frequency of RTEs in the total CD4 T lymphocytes of the group 1 recipients compared with normal donors (P = .009), but not in any of the other groups (Figure 4A). There was no significant difference in the frequency of RTEs in Tcons or Tregs compared with normal donors in any group.
Because the rTregs represent only 20% of the total Tregs, we directly determined the RTE content of the rTregs (Figures 1A and 4B). The frequency and absolute number of RTEs in the rTreg subpopulation of the group 3 recipients with active chronic GVHD was reduced compared with normal donors ($P = .01$), with group 1 recipients with no history of acute or chronic GVHD ($P = .005$), and with group 4 recipients with resolved chronic GVHD ($P = .05$). The percentage of RTEs within the rTregs was the same for all groups and the normal donors (Figure 4B).

To determine whether the decrease in the frequency and number of RTE within the rTreg subpopulation was specific to the rTregs or was related to a general decrease in thymic function, we evaluated the frequency of RTEs in the CD45RA+ Treg subpopulation, which does not express FoxP3 (subpopulation A), as a specificity control (Figures 1A and 4C). The frequency and number of RTEs in subpopulation A T lymphocytes did not differ among the 4 recipients groups and the normal donors, demonstrating that the decreased RTEs in the rTregs of recipients with active chronic GVHD (group 3) was limited to their rTregs.

We performed linear regression analyses of the frequencies of rTregs and their RTEs to evaluate the impact, if any, of the interval after HSCT on immune evaluations. The frequency of rTregs remained stable over time in both the group 1 and group 3 recipients, although the group 3 recipients had consistently lower frequencies (Figure 5A). The group 4 recipients with resolved chronic GVHD had initially elevated rTreg frequencies that decreased over time ($P = .02$). Analyses of RTE frequencies demonstrated stable RTE production in group 1 and 3 recipients over the 12 to 17 year span studied, whereas the group 4 recipients exhibited a...
time-dependent decrease in the frequency of RTEs ($P = .03$) (Figure 5B).

**DISCUSSION**

In our cross-sectional analysis of the role of Tregs in the pathogenesis of chronic GVHD and its clinical resolution in pediatric HSCT recipients, we did not identify any differences in the frequency of immunophenotypic Tregs (CD4$^+$, CD127$^/$CD25$^+$, CD25$^+$) in recipients with or without acute and/or chronic GVHD. However, when using the Miyara classification scheme to focus on the functional Treg subpopulations (rTregs and aTregs), we identified decreases in the frequency and number of rTregs in recipients with active clinical chronic (group 3). The frequencies and number of rTregs in recipients who had had resolution of their chronic GVHD (group 4) or who had no history of either acute or chronic GVHD (group 1)
did not differ from that of normal pediatric bone marrow donors, suggesting that the normalization of rTregs was correlated with the clinical resolution of their chronic GVHD.

HSCT recipients with active chronic GVHD (group 3) and resolved chronic GVHD (group 4) did not differ in any clinical characteristics, including the frequency of previous acute GVHD, use of TBI- or serology-containing preparatory regimens, recipient or donor age, stem cell source, donor–recipient histocompatibility, frequency of female–male donor–recipient pairs, or severity of chronic GVHD. Most importantly, the mean interval from HSCT to immune evaluation did not differ. Previous studies in adult HSCT recipients demonstrated that 50% of recipients with a history of chronic GVHD were completely off immunosuppressive therapy at 7 years [8,9]. Thus, if the recipients with resolved chronic GVHD (group 4) were significantly longer out from HSCT compared with recipients with active chronic GVHD (group 3), then the greater time from HSCT might have contributed to their clinical resolution.

The sole identified difference between the HSCT recipients with active chronic GVHD (group 3) and resolved chronic GVHD (group 4) was the decrease in rTregs in those with active chronic GVHD. The presence of normal frequencies and number of rTregs in recipients with resolved chronic GVHD is consistent with the results of clinical trials with IL-2 in adult HSCT recipients with therapy-resistant chronic GVHD, who demonstrated increased Tregs and clinical improvement [12,13]; however, those adult studies did not assess the effect of IL-2 therapy on the functional Treg subpopulations, either rTregs or aTregs.

Recent studies in murine models of GVHD have demonstrated that acute GVHD results in a marked decrease (>90%) in the frequency of AIRE-expressing (AIRE+) medullary thymic epithelial cells (mTECs) and that the expression of autologous tissue-specific antigens (TSA) is limited in the residual mTECs [14,15]. The reduction in TSA expression was greatest in organs that are the targets of chronic GVHD (eg, gastrointestinal tract, skin, liver). Thus, the reduction in AIRE+ mTECs after acute GVHD can result in both decreased Treg production and the peripheral presence of autoreactive T lymphocytes with potential specificity for chronic GVHD target organs owing to the limited TSA expression and decreased negative thymic selection. Furthermore, following murine acute GVHD, donor-derived T lymphocytes with specificity for shared donor- and recipient-associated antigens have been identified. Potential nonpolymorphic target antigens include TSA, MHC class II determinants, and environmental pathogens [16-19]. Thus, chronic GVHD may be related to the peripheral presence of donor-derived autoreactive T lymphocytes in the context of immune dysregulation (ie, an inadequate number of functional Tregs).

Given that previous clinical evaluations have identified persistent defects in thymic function in HSCT recipients with a history of chronic GVHD, the basis for the normalization of rTregs in the recipients with resolved chronic GVHD was not clear [20,21]. A decreased frequency of apoptotic Tregs was one possibility [22]. Another possibility was that thymic function improved in some recipients with active chronic GVHD, resulting in the production of adequate numbers of rTregs to support normal immune regulation.

Thus, we evaluated the frequency of RTEs (CD4+, CD45RA+, CD31+) in the CD4 T lymphocytes, total Tregs, and the rTreg subpopulation. The frequency of RTEs in the total Tregs was the same in all groups (Figure 4A); however, the frequency and number of RTEs in the rTreg was reduced in recipients with active chronic GVHD (group 3) compared with normal pediatric donors (P = .01) and recipients with resolved chronic GVHD (group 4; P = .05) (Figure 4B). As a specificity control, we measured the frequency and number of RTEs in the CD45RA+ subpopulation of the Tregs that did not express FoxP3 (subpopulation A) (Figure 1A). We found no decrease in the frequency or number of RTEs in the subpopulation A T lymphocytes from any group, demonstrating that the decrease in RTEs in rTregs was limited to the rTreg subpopulation and was not secondary to a general decrease in thymic function.

Our findings of increased/normal RTE frequency in the rTregs of pediatric recipients with resolved GVHD is consistent with the 8-fold increased frequency of RTEs found in the total Tregs of adult chronic GVHD recipients who received low-dose IL-2 and demonstrated clinical improvement [13]. The frequency of T cell excision circles in the total T lymphocytes from adult HSCT recipients (mean age, 57 years) with resolved chronic GVHD was the same as that in age-matched normal controls and superior to that in recipients with active chronic GVHD, suggesting that some adult HSCT recipients have adequate thymic reserves to support the immunologic resolution of their chronic GVHD [23]. Thus, the association of improved thymic function with the clinical resolution of chronic GVHD is not limited to pediatric HSCT recipients.

A significant difference between the recipients with active chronic GVHD (group 3) and those with resolved chronic GVHD (group 4) is that all of the group 3 recipients were on immunosuppressive therapy, whereas most (10 of 14) of the group 4 recipients were off of all therapy and the other 4 recipients were on minimal therapy (Table 4). We believe that the observed differences in the frequencies of both RTEs and rTregs were not related to immunosuppressive therapy, for several reasons: (1) There was no decrease in the frequency or number of RTEs in the subpopulation A T lymphocytes; (2) pediatric patients with juvenile systemic sclerosis who were on and off immunosuppressive therapy had similar decreases in rTregs; and (3) pediatric patients with inflammatory bowel disease who were on immunosuppressive therapy did not have exhibit decreases in rTregs [24 and data not shown]. There were no differences in the frequency of rTregs between the 8 group 3 recipients who were on systemic steroid therapy and the 8 recipients not on systemic steroid therapy (data not shown) Thus, the decrease in rTregs in recipients with active chronic GVHD is not related to their concomitant immunosuppression.

The present report is the first to identify immunologic differences between HSCT recipients with active chronic GVHD and those with resolved chronic GVHD. Previous clinical trials of immunosuppressive therapy to treat chronic GVHD did not explore the biological differences between recipients who were able to discontinue immunosuppressive therapy and those who were not. Our results support the conclusions that the decrease in rTregs in recipients with active chronic GVHD is related to the sustained hypoproduction of rTregs by the thymus, and that the clinical resolution of chronic GVHD and withdrawal of immunosuppressive therapy require the reestablishment of normal immune regulation secondary to the improved thymic production of rTregs.

Debate on the pathogenesis of chronic GVHD has centered on whether chronic GVHD is a continuation of the pathogenic mechanisms that cause acute GVHD (ie, donor-derived T lymphocytes with specificity for recipient-restricted
histocompatibility antigens/cytokines) or a disease of immune dysregulation [1-3]. The present demonstration of decreased rTregs in HSCT recipients with active chronic GVHD is similar to the reported deficiencies of rTregs in both adult and pediatric patients with systemic sclerosis, indicating that immune dysregulation may be central to the pathogenesis of both systemic sclerosis and chronic GVHD [24,25].

The present study has several limitations, principally that it was a cross-sectional study in pediatric patients. A longitudinal study with primarily adult recipients is needed to definitively define the relationship between changes in rTregs and the recipients’ clinical GVHD status, as well as the impact of immunosuppressive therapy on rTregs. The present study was conducted in pediatric HSCT recipients, who have superior thymic function compared with adult HSCT recipients and are less likely to have received mobilized peripheral blood stem cells [26,27]. If the clinical resolution of chronic GVHD is secondary to the thymic generation of peripheral blood stem cells[26,27]. If the clinical resolution of chronic GVHD is secondary to the thymic generation of peripheral blood stem cells, then adult HSCT recipients with lower thymic function might be expected to have lower rates of the immunologic resolution of chronic GVHD. No attempt was made to define the effector mechanisms in the chronic GVHD recipients, which may differ between pediatric and adult HSCT recipients.

Murine experiments have indicated that mTECs are generated from thymic epithelial precursors [28-30]. Thus, HSCT recipients who have experienced decreased AIRE+ mTECs after HSCT and acute GVHD may be able to generate new AIRE+ mTECs if adequate residual TEC precursors are present; however, HSCT recipients who have inadequate residual mTEC precursors may have sustained thymic dysfunction and persistent active chronic GVHD. The clinical administration of IL-2 has produced only temporary increases in Tregs. To achieve sustained improvement in the functional Tregs, future clinical trials should explore the improvement in thymic function after HSCT rather than immunosuppression.

**ACKNOWLEDGMENTS**

The authors thank Manuela Alvarez-Wilson for assistance with manuscript preparation, Renna Killen for obtaining informed consents, and Connie Jackson for data management.

**Financial disclosure:** The authors have nothing to disclose.

**Conflict of interest statement:** There are no conflicts of interest to report.

**Authorship statement:** K.M.M. participated in study design, administration of the clinical protocol, and manuscript review; B.M. performed laboratory evaluations; N.K., A.J.S., and H.A.-A. reviewed clinical data and reviewed the manuscript; R.P. participated in study design, review of medical records, laboratory data review, and manuscript preparation.

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