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Immune Tolerance to Combined Organ and Bone Marrow Transplants After Fractionated Lymphoid Irradiation Involves Regulatory NK T Cells and Clonal Deletion

Masanori Higuchi,* Defu Zeng,* Judith Shizuru,† Jennifer Gworek,† Sussan Dejbakhsh-Jones,* Masaru Taniguchi,‡ and Samuel Strober2*

Immune tolerance to organ transplants has been reported in laboratory animals and in humans after nonmyeloablative conditioning of the host and infusion of donor bone marrow cells. We examined the mechanisms of immune tolerance to mouse cardiac allografts in MHC-mismatched hosts that developed mixed chimerism after posttransplant conditioning with a 2-wk course of multiple doses of lymphoid tissue irradiation, depletive anti-T cell Abs, and an infusion of donor bone marrow cells. When CD1−/− or Jα281−/− hosts with markedly reduced NK T cells were used instead of wild-type hosts, then the conditioning regimen failed to induce tolerance to the heart allografts despite the development of mixed chimerism. Tolerance could be restored to the CD1−/− hosts by infusing enriched T cells from the bone marrow of wild-type mice containing CD1-reactive T cells but not from CD1−/− host-type mice. Tolerance could not be induced in either IL-4−/− or IL-10−/− hosts given the regimen despite the development of chimerism and clonal deletion of host T cells to donor MHC-Ags in the IL-10−/− hosts. We conclude that immune tolerance to bone marrow transplants involves clonal deletion, and tolerance to heart allografts in this model also involves regulatory CD1-reactive NK T cells. The Journal of Immunology, 2002, 169: 5564–5570.

Immune tolerance to skin allografts was first achieved by establishing chimerism after the injection of donor bone marrow cells into neonatal hosts (1). Subsequently, there have been many attempts to induce tolerance to organ allografts in adult laboratory animals with the ultimate goal of developing protocols that have clinical application. Using the approach of combined organ and bone marrow transplantation in hosts conditioned with nonmyeloablative regimens, tolerance has been achieved in rodents, monkeys, mini-swine, and, more recently, in humans (2–7). However, in some studies acceptance of the marrow transplants as judged by the development of mixed chimerism was associated with the rejection of skin grafts or heart grafts (5, 8). Initial reports of radiation chimeras that rejected donor skin grafts suggested that skin-specific transplantation Ags explained the rejection, since the infusion of epidermal cells into chimeras resulted in skin graft acceptance (9, 10).

The above studies may have important implications for clinical transplantation and suggest that acceptance of bone marrow transplants will not necessarily result in the acceptance of other donor tissue transplants due to either tissue-specific transplantation Ags or to differences in the immunogenicity or susceptibility of different tissues to immune rejection. However, there is considerable variability of skin and s.c. myocardial graft acceptance in mixed chimeras depending upon the host-conditioning regimen that is used, the level of donor T cell chimerism achieved, and the time interval between transplantation of the donor bone marrow/hemopoietic progenitor cells and the organ graft (2, 3, 11, 12). In some studies, there is uniform acceptance of these organ grafts (2, 3, 11) and in others the majority of the organ grafts are rejected despite high levels of chimerism (5, 12).

The object of the current study in mice was to elucidate the mechanisms by which mixed chimeras accept or reject s.c. heart grafts using a completely posttransplant-conditioning regimen in which donor bone marrow cells are infused after the transplantation of the heart graft. This posttransplant-conditioning regimen consisting of total lymphoid irradiation (TLI)3 and anti-thymocyte globulin has been previously shown to induce mixed chimerism and tolerance to vascularized heart grafts in completely MHC-mismatched rats (13, 14). A key advantage of the posttransplant regimen is that it can be applied to human cadaver organ transplantation. Because the timing of the availability of cadaver organs cannot be predicted or planned, pretransplant-conditioning regimens cannot be used in the clinical setting. In addition, tolerance induction using the TLI and anti-thymocyte globulin regimen is facilitated by the use of the calcineurin inhibitor, cyclosporine, a frequently used immunosuppressive drug in clinical organ transplantation (14).

We found that the different parts of the host-conditioning regimen, the makeup of the residual host T cell subsets, and host secretion of IL-4 and IL-10 were critical in determining whether tolerance to both the bone marrow and heart grafts was induced. We found a marked increase in the fraction of host T cells that expressed NK cell markers in wild-type recipients, and that the latter T cells, as well as host secretion of IL-4 and IL-10, were required for heart graft acceptance even in mixed chimeras with

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1 Abbreviations used in this paper: TLI, total lymphoid irradiation; ATS, anti-thymocyte serum.
clonal deletion of host T cells. Thus, acceptance of marrow transplants and associated clonal deletion does not ensure the acceptance of organ grafts, and additional mechanisms of tolerance such as immune regulation are required for organ graft acceptance in this model.

Material and Methods

Animals

Wild-type BALB/c (H-2b), C57BL/6 (H-2b), and C3H/He(H-2b) mice were purchased from the Comparative Medicine, Stanford University (Stanford, CA). Male BALB/c IL-4−/− (BALB/c IL-4−/−; IL-4−/−; IL-4−/− mice), BALB/c TNF-α−/− (BALB/c TNF-α−/− mice), and C57BL/6 IL-10−/− (C57BL/6 IL-10−/− mice) were purchased from The Jackson Laboratory (Bar Harbor, ME). Development of CD1−/− founder mice was described previously (15) and those in the current study were maintained on the BALB/c background and kindly provided by Drs. M. A. Extley and S. P. Balk (Harvard University, Boston, MA), and have been backcrossed for more than 10 generations.

Survival of C57BL/6 heart allografts and chimerism in BALB/c hosts given ATS, TLI, and/or donor bone marrow cells

Table 1. Survival of C57BL/6 heart allografts and chimerism in BALB/c hosts given ATS, TLI, and/or donor bone marrow cells

| Host Treatment | Heart Allograft Survival (days) | Median Survival (days) (± SD) | Percentage of Donor Type Cells Amongst White Blood Cells of Host 
|----------------|---------------------------------|------------------------------|----------------------------------
| ATS+ TLI+ bone marrow cells | 9, 14, 14, 14, 17, 17 | 14 (±3) | NA |
| ATS+ bone marrow cells | 25, 27, 29, 33, 34 | 28 (±4) | NA |
| TLI+ bone marrow cells | 13, 14, 17, 20, 45, 48, 55 | 20 (±18) | NA |
| ATS− TLI− bone marrow cells | 38, 45, 48, 49, 50, 52, 55, 59 | 49 (±6) | NA |
| ATS− bone marrow cells | 20, 20, 20, 21, 21, 22, 23 | 21 (±1) | <1, <1, <1, <1, <1, <1, <1 |
| TLI− bone marrow cells | 21, 23, 27, 27, 35, 59, >100 | 27 (±27) | 68, 74, 80, 88, 97, >99, >99 |

a Five doses of 50 µl, i.p. given on days 0, 2, 4, 6, 8 (heart allograft given day 0).
b Ten doses of 240 cGy given on days 1−3, 6−10, 13, and 14.
c A total of 50 × 10⁶ cells injected i.v. on day 15.
d Day 28 after BM cell injection (= day 43 after heart transplantation). Adequate blood samples were not available for analysis in some hosts. NA, not applicable.
NK1.1

Previous studies showed peripheral NK1.1

CD1 study was examined by comparing graft survival in wild-type,

with ATS, TLI, or ATS and TLI before the injection of 50

and left, respectively, of each panel, and percentages in boxes are shown. Hosts were given C57BL/6 heart grafts on day 0 and were conditioned thereafter

with ATS, TLI or ATS and TLI before the injection of 50 \times 10^6\text{ marrow cells}.

White blood cells. When gated donor T cells (H-2K^b^{+}/Thy1.2^{+})

were analyzed for the presence of NK T cells, \(<1\%\) were NK1.1^{+} (data not shown). Similar percentages of donor T and B cells (6.9

and 74.2\%) and granulocytes and monocytes (14.9\%) were found

in hosts given the TLI and marrow cells without ATS (Fig. 1).

Levels of chimerism were stable when reanalyzed at 100 days

(data not shown). Hosts in the group given ATS and donor marrow

cells had \(<0.1\%\) donor-type cells in all lineages tested.

Histopathological studies of heart grafts removed after 100 days

from hosts given the complete TLI, ATS, and bone marrow trans-

plantation regimen showed an intact myocardium with little evi-

dence of myocyte necrosis, mononuclear cell infiltration, scarring,

or hemorrhage (data not shown). In contrast, heart grafts removed

shortly after the cessation of contractions in the other groups

showed intense mononuclear cell infiltration, patchy hemorrhage,

and diffuse myocyte necrosis consistent with acute rejection.

Role of CD1-reactive T cells and cytokines in heart graft acceptance

Recent studies showed that the combined regimen of TLI and ATS

administered to BALB/c or C57BL/6 mice altered the balance of residual T cell subsets such that the minor \((\sim2\%)\) subset of T

cells expressing NK cell markers, DX5^{+}TCR\alpha\beta^{+}, and NK1.1^{+}

TCR\alpha\beta^{+} T cells, became the majority of all T cells (19). These

unusual T cell subsets were regulatory and prevented graft-vs-host

disease (19). The role of CD1-reactive DX5^{+}TCR\alpha\beta^{+} and

NK1.1^{+}TCR\alpha\beta^{+} cells in long-term graft acceptance in the current

study was examined by comparing graft survival in wild-type,

CD1^{-/-}, or J_{\alpha}281^{-/-} hosts given the complete host-conditioning

regimen. Previous studies showed peripheral NK1.1^{+} T cells are

markedly reduced in CD1^{-/-} and J_{\alpha}281^{-/-} mice as compared to

wild-type mice due to either the lack of positive selection by CD1

or the inability to generate the invariant CD-1 reactive \(V_{\alpha}^{+}J_{\alpha}^{+}281\)

TCR\alpha chain, respectively (20–22). In addition, our studies of bone

marrow TCR\alpha\beta^{+} T cells showed that about 20\% of the latter T

cells are NK T cells in wild-type mice, and about 4\% are NK T

cells in CD1^{-/-} mice (21). Less than 1\% of bone marrow T cells that

are CD1-reactive as judged by staining with a CD1-tetramer

reagent loaded with \(\alpha\)-galactosylceramide are present in CD1^{-/-}

mice (data not shown).

Immunofluorescent staining and two-color flow cytometric analysis

of spleen cells for DX5 vs TCR\alpha\beta or NK1.1 vs TCR\alpha\beta mark-

ers was performed in BALB/c and C57BL/6 wild-type mice before

and immediately after treatment with the combined TLI and ATS

regimen without organ and marrow transplants (Fig. 2A). Before

treatment the DX5^{+} and NK1.1^{+} T cells accounted for between

3.6 and 3.7\% of all T cells in wild-type hosts and no discrete

population of cells was observed (enclosed in boxes in Fig. 2A).

At that time point, the mean \pm SD percentage of TCR\alpha\beta^{+} T cells

was 34 \pm 4\% in four wild-type BALB/c mice and 26 \pm 5\% in five

wild-type C57BL/6 mice. After TLI and ATS, the mean percent-

age of T cells in wild-type mice was reduced to 0.4 \pm 0.1

and 0.6 \pm 0.5\%, respectively. The percentage of DX5^{+} and NK1.1^{+} T

cells amongst all residual T cells rose at least 10-fold to about 54

and 38\%, respectively, but the absolute numbers of DX5^{+} and

NK1.1^{+} T cells were slightly reduced as compared to pretreatment

levels (19). The rise in the percentage of DX5^{+} or NK1.1^{+} T cells

amongst all T cells was attenuated after treatment of CD1^{-/-}

BALB/c mice (\sim10\%) and of J_{\alpha}281^{-/-} C57BL/6 mice (\sim5\%) as

compared to that of the wild-type mice (Fig. 2A). The CD1 reac-

tivity of the DX5^{+} and NK1.1^{+} T cells in wild-type BALB/c and

C57BL/6 hosts that received TLI and ATS was confirmed by

>90\% staining positively with CD1 tetramers loaded with

\(\alpha\)-galactosylceramide (data not shown).
Comparison of heart graft survival in CD1−/− BALB/c vs wild-type BALB/c mice given TLI, ATS, and donor marrow cells showed that all grafts were rejected in the CD1−/− group by 95 days, but about 85% of grafts survived >100 days in the wild-type group (p < 0.001 as judged by the log-rank test) (Fig. 2B). Since previous studies (15, 23) indicated that the regulatory functions of NK1.1+ T cells are mediated at least in part by IL-4 or IFN-γ, the survival of heart allografts was compared in IL-4−/−, IFN-γ−/−, and wild-type BALB/c hosts given the complete conditioning regimen. Fig. 2B shows that all heart grafts survived >100 days in the IFN-γ−/− group, but that only about one-third of grafts survived during the same period in the IL-4−/− hosts (p < 0.01 IL-4−/− vs IFN-γ−/−; p < 0.01 IL-4−/− vs wild-type). Untreated IFN-γ−/− hosts rejected heart grafts within the same time interval as untreated wild-type hosts (data not shown).

Table II shows that the use of CD1−/− instead of wild-type BALB/c hosts had an impact on the development of chimerism, since three of six CD1−/− hosts had 1% or less donor-type cells amongst white blood cells (mean 23%). All wild-type hosts had at least 51% donor-type cells (mean 59%) (p < 0.001 by Student’s t test). However, remaining chimeric hosts in the CD1−/− group (23–50% donor-type cells) still rejected their heart allografts despite the complete conditioning regimen. Similarly, six of nine hosts in the IL-4−/− group rejected their heart grafts, and five of six of the latter were chimeras (Table II). Thus, deficiency in CD1 and IL-4 genes had a more robust effect on the rejection of heart as compared to bone marrow allografts.

We theorized that the uniform heart graft rejection observed in CD1−/− hosts was due to a deficiency in CD1-reactive TCRβ− T cells. To reconstitute these T cells in the CD1−/− hosts, we injected them with 0.5 × 10^6 sorted TCRβ− T cells from the bone marrow of wild-type BALB/c mice. Sorted T cells had ≥95% purity as judged by reanalysis. Amongst gated T cells in a wild-type BALB/c bone marrow sample not used for sorting, there were 4.9% CD1-reactive cells as judged by staining with the CD1-tetramer reagent, and there were <0.1% in a CD1−/− marrow sample. Previous studies showed that NK1.1+ T cells depleted from the periphery can be replaced within 48 hr from dividing progenitors in the marrow (24). Because the marrow is shielded during TLI, proliferating cells in the marrow are protected from irradiation. To reduce the possibility that ATS injected into hosts would kill the transferred bone marrow T cells, the conditioning regimen was changed to include only 1 dose of ATS on day 0, and 17 instead of 10 doses of TLI. The BALB/c marrow T cells were injected after 8 doses of TLI. Fig. 2C shows that wild-type BALB/c mice given the regimen of 1 dose of ATS, and 17 doses of TLI and an injection of donor marrow cells all accepted donor C57BL/6 heart grafts for >100 days. These long-term hosts developed immune tolerance, since four hosts given third-party C3H/He heart grafts rejected them within 8–22 days.

CD1−/− BALB/c hosts all rejected the donor C57BL/6 grafts by day 69 (p < 0.001), but CD1−/− hosts given the sorted wild-type marrow TCRβ− T cells all accepted their grafts for at least 100 days (Fig. 2C). A control group of CD1−/− hosts given sorted marrow TCRβ− T cells from CD1−/− BALB/c mice rejected all grafts by day 45. Thus, T cells from wild-type, but not CD1−/− BALB/c, mice prevented the rejection of heart grafts in CD1−/− hosts.

The TCRβ chain of most peripheral CD1-reactive NK1.1+ T cells is derived from an invariant rearrangement of Vβ14 and Jβ281 gene segments (25, 26). To determine whether CD1-reactive cells that regulate heart allograft rejection express the invariant TCRβ chain, the donor and host mouse strains were reversed so...
that wild-type and J<sub>281</sub><sup>–/–</sup> C57BL/6 host mice could be compared for their ability to reject BALB/c heart grafts. The C57BL/6 hosts were conditioned with five doses of ATS, 10 treatments of TLI, and an infusion of 50 × 10<sup>6</sup> BALB/c marrow cells as before. Fig. 2D shows that the C57BL/6 wild-type hosts accepted all BALB/c heart grafts for at least 100 days, and Table II shows that all hosts were mixed chimeras. In contrast, four of seven J<sub>281</sub><sup>–/–</sup> hosts rejected their grafts within 50 days (p < 0.0001) (Fig. 2D). All of the latter hosts were mixed chimeras with between 70 and 78% donor-type cells amongst white blood cells (Table II). The more complete loss of tolerance in the CD1<sup>–/–</sup> as compared to the J<sub>281</sub><sup>–/–</sup> hosts may be due to the presence of CD1-reactive T cells in the latter mice that do not express the V<sub>8</sub>1,281 invariant TCRα chain (27). We also compared the ability of IL-4<sup>–/–</sup> and IL-10<sup>–/–</sup> C57BL/6 hosts with that of wild-type C57BL/6 hosts given the complete conditioning regimen to accept BALB/c heart grafts. The IL-10<sup>–/–</sup> hosts rejected all heart grafts by day 65, and four of six heart grafts were rejected by day 55 by the IL-4<sup>–/–</sup> hosts (Fig. 2D). The graft survival in the IL-10<sup>–/–</sup> and IL-4<sup>–/–</sup> hosts was significantly reduced (p < 0.0001 and p < 0.01, respectively) as compared to wild-type mice. All of the gene-deficient hosts were mixed chimeras (Table II).

**Rejection of second heart grafts in IL-10<sup>–/–</sup> chimeras with clonal deletion**

The development of immune tolerance by host T cells to donor MHC Ags in mixed chimeras has been shown to be due to clonal deletion (28, 29). It was possible that the process of tolerance and clonal deletion in mixed chimeras that rejected heart grafts required several weeks, and that rejection occurred before clonal deletion to donor MHC Ags was complete. This hypothesis was tested by studying IL-10<sup>–/–</sup> chimeric hosts that rejected heart grafts. Six of these hosts received a second donor (BALB/c) heart graft at day 42 (after all first heart grafts had been rejected). All of these hosts had at least 68% donor-type cells amongst white blood cells at day 63 (Table II).

All second heart grafts were rejected within 10 to 28 days (Table III). Chimerism was tested 21 days after the second heart transplantation, and remained in the range of 74–80% donor-type cells (Table III). Chimeras that rejected second heart grafts were tested for clonal deletion of host T cells by staining the spleen cells for the host-type MHC marker (H-2K<sub>b</sub>) vs CD3 and a panel of V<sub>8</sub>1,281 T cells. Gated host T cells (H-2K<sup>b</sup>CD3<sup>+</sup>) were analyzed for the percentage of V<sub>8</sub>2, 3, 4, 5, 6, 9, 11, and 12 cells. Because the percentage of the nondeleted V<sub>8</sub>8 T cells amongst all T cells can vary from group to group, analysis of deleted versus nondeleted V<sub>8</sub> subsets was measured as a ratio of V<sub>8</sub>2,V<sub>8</sub>3, V<sub>8</sub>5, V<sub>8</sub>11, and V<sub>8</sub>12 T cells are deleted (ratio < 0.10) as compared to C57BL/6 splenic T cells as judged by the reduced ratios of these V<sub>8</sub> receptors to that of the nondeleted V<sub>8</sub>8 receptor. The ratios of the V<sub>8</sub>2, V<sub>8</sub>3, V<sub>8</sub>5, and V<sub>8</sub>9 receptors were similar in both strains. The gated host-type (C57BL/6) T cells in the IL-10<sup>–/–</sup> chimeras showed significant reductions in the ratios for the V<sub>8</sub>2, V<sub>8</sub>3, V<sub>8</sub>5, V<sub>8</sub>11, and V<sub>8</sub>12 receptors (p < 0.05 as compared to untreated wild-type C57BL/6 mice. Ratios for V<sub>8</sub>2, V<sub>8</sub>3, V<sub>8</sub>5, and V<sub>8</sub>9 were not significantly different (p > 0.1). Similar reductions in the ratios of V<sub>3</sub>, V<sub>5</sub>, V<sub>11</sub>, and V<sub>12</sub> were found in the C57BL/6 wild-type chimeras that had accepted the heart grafts (Table IV).

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**Table II. Chimerism and survival of allografts in wild-type and gene-deficient hosts given ATS, TLI, and donor bone marrow cells**

<table>
<thead>
<tr>
<th>Host Type</th>
<th>Percentage of Donor-Type Cells Amongst White Blood Cells of Host</th>
<th>Fraction of Hosts with Allograft Surviving &gt;100 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>51, 54, 56, 57, 58, 58, 60, 60, 61, 64, 65</td>
<td>11/13</td>
</tr>
<tr>
<td>CD1&lt;sup&gt;–/–&lt;/sup&gt;</td>
<td>&lt;1, &lt;1, 1, 23, 57, 58</td>
<td>0/8</td>
</tr>
<tr>
<td>IL-4&lt;sup&gt;–/–&lt;/sup&gt;</td>
<td>&lt;1, 53, 57, 60, 65, 67, 70</td>
<td>3/9</td>
</tr>
<tr>
<td>IFN-γ&lt;sup&gt;–/–&lt;/sup&gt;</td>
<td>48, 50, 50, 52, 57, 60, 61, 63, 64, 69</td>
<td>10/10</td>
</tr>
<tr>
<td>C57BL/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>57, 62, 63, 70, 70, 72, 74, 77, 78, 78, 78</td>
<td>12/12</td>
</tr>
<tr>
<td>J&lt;sub&gt;281&lt;/sub&gt;&lt;sup&gt;–/–&lt;/sup&gt;</td>
<td>70, 71, 72, 75, 75, 77, 78</td>
<td>3/7</td>
</tr>
<tr>
<td>IL-4&lt;sup&gt;–/–&lt;/sup&gt;</td>
<td>66, 66, 70, 71, 74, 76</td>
<td>2/6</td>
</tr>
<tr>
<td>IL-10&lt;sup&gt;–/–&lt;/sup&gt;</td>
<td>68, 69, 70, 70, 72, 73, 73, 75, 80, 80</td>
<td>0/10</td>
</tr>
</tbody>
</table>

<sup>a</sup> Day 28 after bone marrow cell injection (= day 43 after heart transplantation). Adequate blood samples were not available for analysis in some hosts.

<sup>b</sup> Second heart allograft given on day 42.

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**Table III. Survival of second BALB/c heart allografts and chimerism in IL-10<sup>–/–</sup> C57BL/6 hosts conditioned with ATS, TLI, and given BALB/c heart and bone marrow transplants**

<table>
<thead>
<tr>
<th>Host Type</th>
<th>Second Heart Allograft Survival&lt;sup&gt;a&lt;/sup&gt; (days)</th>
<th>Percentage of Donor Type Cells Amongst White Blood Cells of Host After Second Allograft Transplantation&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10&lt;sup&gt;–/–&lt;/sup&gt;</td>
<td>10, 10, 13, 22, 22, 28</td>
<td>74, 75, 75, 76, 79, 80</td>
</tr>
</tbody>
</table>
Vp, T cells (p > 0.05). There was a significant reduction (p < 0.05) in the ratio of Vp9 cells in the latter mice as compared to C57BL/6 wild-type mice and the IL-10−/− chimeras, but the ratios differed by <2-fold; in contrast, the ratios of deleted Vp3, 5, and 11 T cells were reduced by at least 5-fold as compared to wild-type mice. Thus, second heart grafts were rejected by the IL-10−/− chimeras despite clonal deletion.

Discussion

The rejection of skin and heart allografts by mixed chimeras has been reported previously in mice, dogs, and mini-swine (5, 8, 12). In the current study, a posttransplant host-conditioning regimen of fractionated lymphoid irradiation, depletive anti-T cell Abs, and an infusion of donor bone marrow cells allowed BALB/c or C57BL/6 wild-type mice to develop mixed chimerism and tolerance to MHC-mismatched heart grafts placed in the ear pinnae. This regimen has been used successfully to induce tolerance to vascularized heart allografts in rats and to kidney allografts in humans (7, 13, 14). Unexpectedly, the removal of the anti-T cell Abs from the regimen in the current study resulted in uniform heart graft rejection with uniform mixed chimerism.

We analyzed the role of residual host NK1.1+ and/or DX5+ T cells in the development of tolerance to the heart grafts. NK1.1+ T and DX5+ T cells are markedly increased in hosts given TLI, but do not increase significantly after sublethal total body irradiation (19). More than 90% of these C57BL/6 and BALB/c host T cells were reactive to CD1 and expressed the NK T cell invariant TCRa chain as judged by positive staining with a CD1 tetramer loaded with the a-galactosylceramide ligand (data not shown). NK1.1+ T cells have been shown to facilitate tolerance to tissue allografts after costimulatory blockade (23), prevent graft-vs-host disease (18, 19), and facilitate tolerance to heterologous proteins in the anterior chamber autoimmune eye disease model (30). The critical role of the host NK1.1+ and DX5+ T cells in the current study was shown by the loss of tolerance to heart grafts in the CD1−/− or J1.281−/−-deficient hosts as compared to wild-type hosts, and the ability to reconstitute long-term heart graft acceptance by the transfer of wild-type host bone marrow T cells containing CD1−/− reactive T cells, but not by the transfer of CD1−/−/− marrow T cells. Although the C57BL/6 donor marrow cells contained about the same level of CD1-tetramer+ T cells as the BALB/c marrow cells (data not shown), the CD1-reactive T cells contained in the donor marrow (injected after TLI) did not allow for graft acceptance, and donor-type NK T cells could not be detected in the spleen on day 28 after transplantation. Injection of host marrow T cells was done during rather than after TLI. Long-term heart graft acceptance was markedly reduced in the IL-4−/− and IL-10−/−, but not in the IFN-γ−/−, hosts. The results suggest that secretion of both IL-4−/− and IL-10−/− by CD1-reactive NK T cells and/or conventional T cells in the hosts facilitates tolerance induction to the heart grafts. We did not demonstrate that the IL-4 and IL-10 were secreted by the CD1-reactive NK T cells in the current study, but our previous studies showed that the regulatory function of enriched NK T cells was lost when obtained from IL-4−/− mice (18). Thus, the latter cells are the likely source of the cytokines required for tolerance. However, the NK T cells may polarize other host or donor T cells toward a Th2-immune response. Thus, tolerance may require IL-4 and/or IL-10 secretion by both NK and non-NK T cells. Determination of the contribution of T cell subsets to cytokine secretion is the subject of a separate study. Despite the failure of heart graft acceptance, IL-10−/− hosts developed uniform mixed chimerism associated with clonal deletion of C57BL/6 host T cells. Yet, second BALB/c heart grafts were rejected within 28 days, despite the high levels of chimerism measured at 21 days.

The clonal deletion pattern of the host Vp, T cell subsets indicates that the C57BL/6 (H-2b) host cells have lost reactivity to donor (H-2d) MHC Ags. Previous studies of mixed chimeras have shown that negative selection of host T cells is due to the presence of donor-derived dendritic cells in the host thymus (28). However, the ability of the chimeric hosts to reject the heart, but not bone marrow, grafts indicated that tissue-specific minor, non-MHC, transplantation Ags are likely expressed by the donor heart cells but are not expressed by the donor bone marrow cells or their progeny. Presumably neither host nor donor-derived dendritic cells in the thymus express these heart-specific minor Ags which are the likely targets of rejection in some chimeric hosts. Reactivity to the latter Ags and associated rejection appears to be prevented by the regulatory cells and the secretion of IL-4 and IL-10.

In conclusion, clonal deletion of host T cells to donor MHC Ags was insufficient to achieve tolerance to the heart grafts. Other tolerance mechanisms were involved that included the contribution of regulatory CD1-reactive T cells with NK cell markers that secrete high levels of IL-4 and IL-10. We previously called these regulatory T cells from TLI-treated mice “natural suppressor” cells (31). The results impact on clinical organ transplantation and suggest that acceptance of bone marrow transplants from an organ donor and associated clonal deletion will not result in uniform organ graft acceptance unless additional mechanisms of immune tolerance are in place.

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References


