Dissociation of Hemopoietic Chimerism and Allograft Tolerance After Allogeneic Bone Marrow Transplantation

Akihisa Umemura, Hirofumi Morita, Xian Chang Li, Steven Tahan, Anthony P. Monaco and Takashi Maki

J Immunol 2001; 167:3043-3048; doi: 10.4049/jimmunol.167.6.3043
http://www.jimmunol.org/content/167/6/3043

Why The JI?

• Rapid Reviews! 30 days* from submission to initial decision
• No Triage! Every submission reviewed by practicing scientists
• Speedy Publication! 4 weeks from acceptance to publication

*average

References

This article cites 31 articles, 10 of which you can access for free at:
http://www.jimmunol.org/content/167/6/3043.full#ref-list-1

Subscription

Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Dissociation of Hemopoietic Chimerism and Allograft Tolerance After Allogeneic Bone Marrow Transplantation

Akihisa Umemura,* Hirofumi Morita,* Xian Chang Li, † Steven Tahan, ‡ Anthony P. Monaco,* and Takashi Maki2*†

Creation of stable hemopoietic chimerism has been considered to be a prerequisite for allograft tolerance after bone marrow transplantation (BMT). In this study, we demonstrated that allogeneic BMT with bone marrow cells (BMC) prepared from either knockout mice deficient in both CD4 and CD8 T cells or CD3E-transgenic mice lacking both T cells and NK cells maintained a high degree of chimerism, but failed to induce tolerance to donor-specific wild-type skin grafts. Lymphocytes from mice reconstituted with T cell-deficient BMC proliferated when they were injected into irradiated donor strain mice, whereas lymphocytes from mice reconstituted with wild-type BMC were unresponsive to donor alloantigens. Donor-specific allograft tolerance was restored when donor-type T cells were adoptively transferred to recipient mice given T cell-deficient BMC. These results show that donor T cell engraftment is required for induction of allograft tolerance, but not for creation of continuous hemopoietic chimerism after allogeneic BMT, and that a high degree of chimerism is not necessarily associated with specific allograft tolerance. The Journal of Immunology, 2001, 167: 3043–3048.

Complete depletion of donor T cells from bone marrow cell (BMC)3 preparations is often associated with failure of donor bone marrow engraftment following allogeneic bone marrow transplantation (BMT), suggesting that some T cells may facilitate donor bone marrow engraftment and creation of stable chimerism (1–5). Moreover, it has been suggested that a correlation exists between high levels of initial donor T cell reconstitution and stable chimerism, which is considered to be a prerequisite for allograft tolerance in the radiation-based mixed allogeneic chimera model (6). The role of chimerism in allograft tolerance induction by nonradiation-based protocols is controversial (7–9). In this study, we demonstrate that dissociation of hemopoietic chimerism and allograft tolerance, i.e., absence of donor-specific allograft tolerance despite continuous chimerism, occurs after allogeneic BMT with T cell-deficient BMC. Requirement of T cells for achieving donor-specific allograft tolerance was confirmed by restoration of donor-specific allograft tolerance after adoptive transfer of donor T cells to mice given T cell-deficient bone marrow transplants.

Materials and Methods

Mice

B10.A (H-2b) mice (Charles River Laboratories, Kingston, NY) and B10.D2 (H-2b) mice (The Jackson Laboratory, Bar Harbor, ME) were used as BMC recipients. BMC donors included wild-type (wt) C57BL/6 (wtB6; H-2b/k) mice (The Jackson Laboratory, Bar Harbor, ME) and B10.A (H-2a) mice (Charles River Laboratories, Kingston, NY) and B6.CBAF1 (H-2b/k) mice carrying a human CD3E transgene (CD3E-tg mice) (13). T cell-deficient mice, their wt controls, and DBA/1 mice were purchased from The Jackson Laboratory. BMT recipients were killed 81–83 days after skin grafting (111–113 days after BMT). All care and handling of animals was conducted in accordance with guidelines provided in the Guide for Care and Use of Laboratory Animals published by the U.S. Department of Health and Human Services.

Preparation of radiation chimeras

Recipient mice were irradiated with a single dose of 6.5 or 7.5 Gy from a Cs source (Nordion, Ontario, Canada). BMC were harvested from the femurs and humeri of donor mice by flushing with HBSS. Wild-type BMC as well as CD4 KO and CD8 KO BMC were incubated with rat anti-mouse Thy-1.2 mAb (clone 53-2.1; BD PharMingen, San Diego, CA) and immunomagnetic beads conjugated with goat anti-rat IgG (Dynabeads M-450; Dynal, Lake Success, NY) before BMT to prevent graft-vs-host disease. T lymphocytes from mice reconstituted with 25 × 10^6 allogeneic BMC 4–6 h after irradiation were adoptively transferred to recipient mice given T cell-deficient bone marrow transplantation.

Skin grafting

Full-thickness skin grafts were transplanted onto the lateral thoracic area of the recipients using standard techniques 30 days after BMT, as described previously (14).

Flow cytometry

The cells were incubated with an anti-CD16/32 mAb for 10 min to block nonspecific binding of labeled Abs. Splenocytes were stained with the FITC-, PE-, CyChrome-conjugated Abs directed to H-2Kb, H-2Kb, CD4, CD8a, CD11b (macrophages/monocytes), CD11c (dendritic cells), and CD45R (B cells) (BD Pharmingen). FITC-, PE-, CyChrome-conjugated isotype Abs were used as controls. Stained cells were analyzed on a FACScan (BD Biosciences, Mountain View, CA). Donor cell chimerism was determined by flow cytometric analysis of recipient peripheral and/or splenic lymphoid cells with normal donor- and recipient-type cells as positive and negative controls. The percentage of chimeric H-2Kb-positive cells was calculated using the formula: 100 (net percentage in the test samples) – (net percentage in the negative control samples)) / (net percentage in the positive control samples) – (net percentage in the negative control samples)). Net percentage refers to the percentage obtained after subtraction of staining with the appropriate isotype controls.
In vivo assay for T cell proliferation

Lymphocytes were isolated from spleens and peripheral lymph nodes of B10.A recipients 28 days after BMT (without skin grafting), and labeled with CFSE (Molecular Probes, Eugene, OR), as described previously (15). CFSE-labeled cells (40–60 x 10^7) were injected through the tail vein into corresponding syngeneic mice (B10.A), wt BMC donor mice (B6 or B6129SF2), and third-party mice (DBA/1), all of which were lethally irradiated (10 Gy). In the case of lymphocytes prepared from B10.D2 mice transplanted with CD3ε-tg BMC, they were injected into lethally irradiated B10.D2 (syngeneic), wtB6CBAF3 (donor-specific), or DBA/1 (third-party) mice. Lymphoid cells prepared from naive B10.A mice or B10.A mice sensitized to wtB6 by skin grafting were also labeled with CFSE and injected into irradiated B10.A, wtB6, or DBA/1 one. One to four days after adoptive transfer of CFSE-labeled cells, a single-cell suspension of spleens was prepared and stained with PE-conjugated anti-CD4 mAb and analyzed on a FACScan. Injected CD4^+ T cells were identified in the CFSE^–D^+ gate. The frequency of proliferating CD4^+ T cells was calculated as described previously (16).

Reconstitution with donor-type T cells

B10.A mice were lethally (9.5 Gy) irradiated and reconstituted with 25 x 10^6 T cell-depleted wtB6 BMC. Maturation of donor T cells was usually seen 30 days after reconstitution and stabilized by 60 days (14). Splenic and lymph node lymphocytes harvested 45 days after BMT were enriched for T cells (>85%) by a nylon wool column separation. Flow cytometric analysis showed that >95% of T cells were of donor type. Enriched T cells (15 x 10^6) were injected i.v. into CD4/8 DKO BMC-reconstituted B10.A mice 21 days after BMT. The presence of injected donor-type T cells was determined by flow cytometry on day 28 after BMT (7 days after T cell transfer). The mice were transplanted with wtB6129SF2 skin grafts on day 30 after BMT.

Results

Effect of radiation dose on induction of chimerism and allograft tolerance

Transplantation of 25 x 10^6 T cell-depleted wtB6 BMC in sublethally irradiated (7.5 Gy) B10.A mice achieved varying degrees of hemopoietic chimerism on day 28 after BMT (Table I, group a). The majority (11 of 12) of mice showed chimerism of >88% and remained highly chimeric on day 90 in all lymphoid cell types, including CD4^+ and CD8^+ T cells, B cells, macrophages/monocytes, and dendritic cells (Table II). These chimeric mice accepted wtB6 skin grafts transplanted on day 30 (Table III, group a). In contrast, B10.A mice irradiated at 6.5 Gy and given 25 x 10^6 T cell-depleted wtB6 BMC (wtB6/6.5Gy BMC recipients) failed to achieve either chimerism or allograft tolerance. Flow cytometric analyses on day 28 showed a lower degree of chimerism (26%) as compared with wtB6/7.5Gy BMC recipients (87%; Table I, group b). Donor-type skin grafts placed on day 30 were rejected within 15 days (Table III, group b). The degree of chimerism decreased progressively without corresponding to the timing of graft rejection, and by day 90 none of the recipient mice were chimeric. As both CD4^+ and CD8^+ donor-type T cells were not detected in these mice on day 28 despite the presence of other donor-type lymphoid cells, we examined whether donor T cell reconstitution is essential in establishment of chimerism and allograft tolerance in this model.

Chimerism without allograft tolerance after transplantation of T cell-deficient BMC

When 25 x 10^6 BMC prepared from CD4 KO or CD8 KO mice were transplanted in sublethally irradiated (7.5 Gy) B10.A mice, a high degree of chimerism (Table I, groups c and d) was maintained for at least 90 days, correlating with donor-specific allograft tolerance (Table III, groups c and d). Loss of chimerism in these mice was always associated with graft rejection, as seen in wtB6/7.5Gy BMC recipients. However, when BMC prepared from CD4/8 DKO mice were transplanted to sublethally irradiated (7.5 Gy) B10.A recipients, the outcome was strikingly different. Five of seven mice promptly rejected wtB6129SF2 skin allografts even in the presence of a high-degree donor chimerism (Table I, group e, and Table III, group f). The dissociation of hemopoietic chimerism and allograft tolerance was also observed with the use of BMC from CD3ε-tg mice, which are deficient in all T cells and NK cells (13) (Table I, group f, and Table III, group h). On day 28 after BMT, the degree of chimerism was moderate, with approximately one-half of B10.D2 (H-2^d) recipients showing <50% chimerism. On day 90, 5 of 11 mice showed a high-degree chimerism (80–95%), while chimerism disappeared in the rest of the mice. Again, donor-type (wtB6CBAF3) skin grafts were acutely rejected not only in mice that lost chimerism, but in mice that maintained a high degree of chimerism. Transplantation of wt B6129SF2 or B6CBAF3 BMC induced both a high degree of chimerism and acceptance of corresponding skin grafts (Table III, groups e and g). Thus, induction of donor-specific allograft tolerance, but not continuous chimerism, required donor T cell reconstitution. Moreover, highly chimeric mice were capable of rejecting donor-specific skin allografts in this model.

Histology of skin grafts

Histology of the rejecting skin grafts in mice given BMC from CD4/8 DKO mice (Fig. 1B) revealed intense cellular infiltration.

Table I.  Chimerism after BMT

<table>
<thead>
<tr>
<th>Group</th>
<th>BMC Donor</th>
<th>Dose of Radiation (Gy)</th>
<th>Assay Days</th>
<th>No. of Mice Chimeric/Total</th>
<th>Chimerism, % Donor Cells (range)</th>
<th>CD4^+ T Cells</th>
<th>CD8^+ T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>wtB6</td>
<td>7.5</td>
<td>28</td>
<td>12/12</td>
<td>86.6 ± 5.3 (28.4–95.9)</td>
<td>3.9 ± 0.5</td>
<td>5.9 ± 1.2</td>
</tr>
<tr>
<td>b</td>
<td>wtB6</td>
<td>90</td>
<td>11/12</td>
<td>97.0 ± 0.4 (94.6–97.9)</td>
<td>9.1 ± 0.6</td>
<td>1.7 ± 0.3</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>c</td>
<td>CD4 KO</td>
<td>7.5</td>
<td>28</td>
<td>0/12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>d</td>
<td>CD8 KO</td>
<td>7.5</td>
<td>28</td>
<td>10/10</td>
<td>95.7 ± 0.9 (91.4–99.1)</td>
<td>1.6 ± 0.3</td>
<td>4.2 ± 2.0</td>
</tr>
<tr>
<td>e</td>
<td>CD4/8-DKO</td>
<td>7.5</td>
<td>28</td>
<td>9/10</td>
<td>94.3 ± 0.7 (91.3–97.1)</td>
<td>10.4 ± 1.0</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>f</td>
<td>CD3ε-tg</td>
<td>7.5</td>
<td>28</td>
<td>10/11</td>
<td>54.1 ± 12.7 (3.9–91.3)</td>
<td>17.4 ± 4.2</td>
<td>10.7 ± 2.3</td>
</tr>
</tbody>
</table>

* B10.A (groups a–e) or B10.D2 (group f) mice were irradiated at a dose indicated above and transplanted with 25 x 10^6 BMC prepared from wtB6 or various T cell-deficient mice. Splenic cells were analyzed for chimerism at 28 and 90 days after BMT. Chimeric mice were used to calculate percentage of donor cells because some mice failed to create chimerism or subsequently lost chimerism. Donor and host CD4^+ and CD8^+ T cells were expressed as percentages in the gated cells.
consisting predominantly of neutrophils, monocytes/macrophages, and rare small lymphocytes forming a bandlike infiltrate in the plane along the dermal-s.c. tissue interface. The epidermis exhibited ischemic changes with areas of ulceration and outward elimination of degenerate reticular dermal collagen into the overlying crust. A similar picture was seen in rejecting skin grafts in mice given CD3E-tg BMC (data not shown). Skin grafts in wtB6/6.5Gy BMT recipients (Fig. 1A) showed more cellular infiltration, with a high number of neutrophils mixed with monocytes/macrophages and rare small lymphocytes. Skin grafts in tolerant mice at 140 days showed no cellular infiltration (Fig. 1C).

**In vivo mixed lymphocyte responses**

To quantitatively analyze the host T cell reactivity to alloantigens after allogeneic BMT, lymphocytes harvested at day 28 were labeled with a fluorochrome and CFSE and injected into lethally irradiated (10 Gy) stimulator mice. Because CFSE segregates equally between two daughter cells with each cell division, the CFSE profile of T cells recovered from the stimulator mice correlates with the degree of proliferation by the adoptively transferred host T cells against alloantigens of the stimulator mice (15–17) (in vivo MLR).

As shown in Fig. 2A, CD4+ T cells of naive B10.A mice proliferated at least six times by day 3 after injection in B6 stimulator mice with 9.5% of CFSE-labeled CD4+ T cells proliferating. The same T cells also proliferated in response to DBA/1 alloantigens with responder frequency of 11.3%, while they exhibited minimum proliferation in syngeneic B10.A mice (2.8%). More T cells responded to stimulator alloantigens 4 days after adoptive transfer (Fig. 2C). T cells prepared from B10.A mice given 7.5 Gy and wtB6 BMC failed to proliferate in wtB6 hosts (responder frequency of 2% on day 3), suggesting that they were unresponsive to B6 alloantigens. The same T cells proliferated in the third-party DBA/1 hosts as vigorously as naive B10.A T cells (Fig. 2, B and C). Similarly, T cells from B10.A mice reconstituted with CD4 KO BMC or CD8 KO BMC failed to respond to B6 alloantigens, but proliferated in response to DBA/1 alloantigens (data not shown). In contrast, T cells from B10.A mice given CD4/8 DKO BMC or B10.D2 mice given CD3E-tg BMC proliferated in B6129SF2 or B6CBAF1 stimulator mice, respectively, with responder frequencies of 10.3 and 10.1%, respectively, suggesting the presence of the host T cell clones reactive to stimulator alloantigens. T cells from wtB6/6.5Gy BMT recipients (B10.A) proliferated more in B6 hosts with greater frequency (12%), although the response was not as vigorous as proliferation by T cells of B10.A mice sensitized to B6 alloantigen (18.2%). The kinetics of in vivo proliferation is summarized in Fig. 2C. Thus, in vivo proliferative response by host T cells strongly correlated with the fate of skin allografts.

**Restoration of allograft tolerance by adoptive transfer of donor T cells**

To confirm that donor-type T cells are indeed required for induction of allograft tolerance after BMT, we adoptively transferred donor-type T cells to B10.A mice 21 days after CD4/8 DKO BMT. T cells were prepared from the spleens and lymph nodes of lethally

**Table II. Chimerism 90 days after allogeneic BMT**

<table>
<thead>
<tr>
<th>Group</th>
<th>BMC Donor</th>
<th>Total Lymphoid Cells</th>
<th>CD4+ T Cells</th>
<th>CD8+ T Cells</th>
<th>B Cells</th>
<th>Macrophages/Monocytes</th>
<th>DC Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>wtB6</td>
<td>9.0 ± 0.6</td>
<td>8.61 ± 4.0</td>
<td>9.34 ± 2.1</td>
<td>9.75 ± 0.4</td>
<td>9.39 ± 1.0</td>
<td>100 ± 0.0</td>
</tr>
<tr>
<td>b</td>
<td>CD4 KO</td>
<td>0 ± 0.6</td>
<td>9.55 ± 1.7</td>
<td>9.52 ± 0.7</td>
<td>9.03 ± 0.3</td>
<td>9.98 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>CD8 KO</td>
<td>9.0 ± 1.1</td>
<td>9.0 ± 2.3</td>
<td>9.11 ± 0.9</td>
<td>86.8 ± 0.4</td>
<td>99.0 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>CD4/8-DKO</td>
<td>8.87 ± 2.5</td>
<td>9 ± 0</td>
<td>9.45 ± 1.7</td>
<td>85.7 ± 1.5</td>
<td>99.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>CD3E-tg</td>
<td>9.16 ± 0.6</td>
<td>0 ± 0</td>
<td>9.56 ± 0.5</td>
<td>85.1 ± 0.3</td>
<td>99.2 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

* B10.A (groups a–e) or B10.D2 (group f) mice were irradiated and transplanted with 25 × 10^6 BMC prepared from wtB6 or various T cell-deficient mice. Splenic cells were analyzed 90 days after BMT for phenotypes of chimeric cells and the proportions of each cell type within chimeric cells.

† Percentage of donor-derived cells.

‡ Chimerism was absent in all mice.

**Table III. Skin graft survival after BMT**

<table>
<thead>
<tr>
<th>Group</th>
<th>BMC Donor</th>
<th>Radiation Dose (Gy)</th>
<th>Skin Donor</th>
<th>n</th>
<th>Skin Graft Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>wtB6</td>
<td>7.5</td>
<td>wtB6</td>
<td>12</td>
<td>9, &gt; 80 × 11</td>
</tr>
<tr>
<td>b</td>
<td>wtB6</td>
<td>6.5</td>
<td>wtB6</td>
<td>6</td>
<td>8, 8, 9, 9, 10, 15</td>
</tr>
<tr>
<td>c</td>
<td>CD4 KO</td>
<td>7.5</td>
<td>wtB6</td>
<td>12</td>
<td>8, 11, &gt; 80 × 10</td>
</tr>
<tr>
<td>d</td>
<td>CD8 KO</td>
<td>7.5</td>
<td>wtB6</td>
<td>10</td>
<td>11, &gt; 80 × 9</td>
</tr>
<tr>
<td>e</td>
<td>B6129SF2</td>
<td>7.5</td>
<td>B6129SF2</td>
<td>6</td>
<td>22, 33, 44, &gt; 80 × 3</td>
</tr>
<tr>
<td>f</td>
<td>CD4/8-DKO</td>
<td>7.5</td>
<td>B6CBAF1</td>
<td>9</td>
<td>8, 8, 9, 9, 14, 18, 28, &gt; 80 × 2</td>
</tr>
<tr>
<td>g</td>
<td>B6CBAF1</td>
<td>7.5</td>
<td>B6CBAF1</td>
<td>8</td>
<td>11, 15, 60, &gt; 80 × 5</td>
</tr>
<tr>
<td>h</td>
<td>CD3E-tg</td>
<td>7.5</td>
<td>B6CBAF1</td>
<td>11</td>
<td>8, 8, 8, 9, 9, 9, 9, 12, 16</td>
</tr>
</tbody>
</table>

* B10.A (groups a–f) or B10.D2 (groups g and h) mice were irradiated at a dose indicated above and reconstituted with 25 × 10^6 BMC prepared from wt or various T cell-deficient mice. Skin grafts from wt mice of BMC donors were transplanted 30 days after BMT.

† Numbers in bold font indicate graft survival in mice with stable chimerism.

‡ Indicates that neither CD4+ nor CD8+ T cells of donor origin were detected by flow cytometry on day 28.
irradiated B10.A mice given T cell-depleted wtB6129SF1 BMT. T cells harvested 45 days after BMT were >95% donor (B6129SF1) type by flow cytometric analysis and unresponsive to B10.A alloantigen in vivo and in vitro (data not shown). Fig. 3A shows that donor-type T cells were present 7 days after adoptive transfer (28 days after BMT) in mice reconstituted with CD4/8 DKO BMC, whereas no donor-type T cells were detected without adoptive transfer. The mice given donor T cells were transplanted with wtB6129SF1 skin grafts 30 days after BMT (9 days after adoptive transfer). All skin grafts were accepted for 140 days (Fig. 3B). Flow cytometric analysis on day 102 show a high degree of chimerism (>90%) in all mice (data not shown). Thus, engraftment of donor-type T cells restored allograft tolerance in T cell-deficient BMT recipients by down-regulating the immune response directed against their own alloantigens.

**Discussion**

When low-dose irradiation (6.5 Gy) was given at the time of BMT (wtB6/6.5Gy BMT recipients), some host T cells survived from radiation and caused rejection of both chimeric cells and skin grafts. Flow cytometric analysis 28 days after BMT showed higher percentages of host T cells in the peripheral blood of wtB6/6.5Gy BMT recipients than in the blood of mice irradiated at 7.5 Gy. High percentages of host T cells were also observed in mice given...
CD4/8 DKO or CD3E-tg BMT under 7.5 Gy irradiation. CD4+ T cells from wtB6/6.5Gy BMT recipients and CD4+ T cells from CD4/8 DKO BMT or CD3E-tg BMT recipients (7.5 Gy) proliferated in response to BMC donor alloantigens in in vivo MLR assays, suggesting that they were reactive to donor alloantigens. In contrast, CD4+ T cells from wtB6/7.5Gy BMT recipients were unresponsive to donor alloantigens. Why did T cell-deficient BMT recipients reject skin grafts, but not chimeric donor cells, while wtB6/6.5Gy BMT recipients rejected both skin grafts and chimeric cells? Involvement of skin-specific Ag (18, 19) was ruled out as cause of skin graft rejection because the degree of in vivo proliferation correlated well with specific skin allograft survival and adoptive transfer of wt donor T cells alone without exposure to skin Ag-restored tolerance to skin allografts. In vivo MLR showed that the responder frequency of donor-reactive CD4+ T cells was smaller in T cell-deficient BMC recipients than that in wtB6/6.5Gy BMT recipients. Histology of skin grafts in wtB6/6.5Gy BMT recipients as well as CD4/8 DKO or CD3E-tg BMC recipients showed features characteristic of delayed-type hypersensitivity response, with massive infiltration by inflammatory cells consisting of mostly neutrophils and macrophages (20–22). Together, we postulate that the size of the donor-reactive CD4+ T cell clone in T cell-deficient BMC recipients was large enough to recruit and activate inflammatory cells within the skin grafts, leading to graft rejection, but not sufficient to generate cytotoxic CD8+ effector T cells that may be required for rejection of donor hemopoietic cells. Activated inflammatory cells, including macrophages and neutrophils, contribute to graft damage through production of several toxic molecules such as NO, TNF-α, and hydrolytic enzymes.

Although B10.A mice reconstituted with CD4/8 DKO BMC acutely rejected wt (B6129SF2) skin allografts, adoptive transfer of donor wt T cells in these mice led to acceptance of skin grafts, suggesting that donor T cells were essential in achieving allograft tolerance. Adoptively transferred T cells were derived from lethally irradiated hosts (B10.A) given T cell-depleted wt (B6129SF2) BMC. Thus, the T cells were mostly of B6129SF2 origin, but incapable of causing graft-vs-host response upon transfer to B10.A hosts. These donor T cells contained immunoregulatory T cells that were capable of inhibiting the host T cell response directed against their own (donor) alloantigens. Results of skin graft survival suggest that these immunoregulatory T cells differentiate from wt, CD4 KO, or CD8 KO BMC, but not from CD4/8 DKO or CD3E-tg BMC. As maturation of CD8+ or CD4+ T cells is unaffected in CD4 or CD8 KO mice, respectively, it is possible that both CD4+CD8+ and CD4+CD8- T cells are capable of exerting immunoregulatory activity. Alternatively, as two mice given CD4/8 DKO BMC accepted wt skin grafts, CD4-8+ αβ-TCR-positive cells derived from CD4/8 DKO BMC (23) may function as immunoregulatory cells. Complete lack of allograft tolerance in mice reconstituted with CD3E-tg BMC is probably attributable to more complete loss of T cells through deficiency in early T cell differentiation by CD3-e gene disruption (13). It is not known whether the immunoregulatory T cells identified in the present study are the same as previously described tolerance-inducing veto cells or bone marrow facilitator cells (5, 24–30).

We have previously reported that rejection of donor-type skin allografts in the presence of a high degree of hemopoietic chimerism occurs following allogeneic BMC with MHC class II Ag-deficient BMC (14). Skin allografts showed delayed-type hypersensitivity-like histology (A. Umemura, unpublished observation). Taken together, the present results suggest that chimerism and allograft tolerance are probably two interrelated, but distinct events. They are interrelated because allogeneic BMC that achieves thymic clonal deletion by donor class II Ag-bearing cells (31, 32) and peripheral immunoregulation by donor T cells, and/or full deple- tion or inactivation of host peripheral T cells by the strong immunoablative regimens induces tolerance to donor alloantigen, leading to both durable chimerism and allograft tolerance. They are also distinct because donor skin graft rejection does not lead to rejection of chimerism as long as the clonal size of donor-reactive T cells remains too small to generate direct cytotoxicity against donor lymphoid cells. It is possible that chimerism could be lost at a later time after the end of the observation period (110 days after BMT or 80 days after skin grafting) in mice given CD4/8 DKO or
Dissociation of chimerism and allograft tolerance

CD3E-tg BMT. As skin graft rejection took place 8–28 days (mostly within 14 days) after transplantation, and a high degree (average 90%) of chimerism continued for at least 30 days or more in these mice, these results would still argue for the dissociation of chimerism and allograft tolerance in these mice.

Thus, in the allogeneic BMT model, presence of chimerism may not necessarily guarantee allograft tolerance, particularly when residual host T cells are not completely inactivated or deleted. On the other hand, absence or loss of chimerism after BMT is caused by a large residual host T cell clone and possibly newly developing host T cells, and is usually associated with absence/loss of tolerance.

References


