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The Abrogation of Allosensitization Following the Induction of Mixed Allogeneic Chimerism

Yolonda L. Colson,* Matthew J. Schuchert, † and Suzanne T. Ildstad‡

The association of preformed anti-donor Abs with the hyperacute rejection of bone marrow and solid organ allografts and the persistence of the anti-donor immune response secondary to immunologic memory make allosensitization an absolute contraindication to transplantation. Mixed allogeneic (A + B→A) bone marrow chimeraism has been demonstrated to confer donor-specific tolerance in nonsensitized recipients, but has not been evaluated in the setting of allosensitization. The current study documents that despite significant anti-donor sensitization, mixed allogeneic engraftment is possible and provides a marked advantage over fully allogeneic (B→A) models. Moreover, the acceptance of donor skin grafts and loss of circulating anti-donor Abs suggest that allosensitization can be abrogated with the induction of stable mixed allogeneic chimerism. The Journal of Immunology, 2000, 165: 637–644.

I mproved immunosuppressive agents and technical advances have made transplantation a clinical reality. However, a subset of patients is denied transplantation due to the presence of circulating anti-donor Abs. With over 20% of potential renal transplant candidates, and up to 85% of heavily transfused patients with hemoglobinopathies, exhibiting significant amounts of anti-HLA Abs in their sera, sensitization of recipients to donor alloantigens is a major limitation to both solid organ and bone marrow (BM) transplantation (BMT) (1–4). The presence of Ab toward >60% of lymphocytes in a mixed panel of HLA phenotypes makes finding a suitable organ donor highly unlikely. In aplastic anemia, alloantibodies increase the rate of graft failure up to 40% if the Ab specificities are directed against donor alloantigens (3–5). However, graft failure following sensitization involves more than high titters of circulating anti-donor Abs. Aggressive protocols involving plasmapheresis, immunoabsorption, and/or antithymocyte globulin (with or without radiation and cyclophosphamide conditioning) have failed to significantly change the clinical picture for these sensitized patients, principally due to immunologic (cellular) memory and the eventual return of anti-donor Abs (4–6). In cases requiring BMT or vital solid organs, in which other life-saving therapies are not available, many patients die before a negative donor becomes available. It would be of obvious clinical benefit if such allosensitization could be abrogated and permanent donor-specific tolerance achieved in the setting of BMT.

Mixed allogeneic chimeraism, induced by the reconstitution of lethally irradiated animals with a mixture of host- and donor-type BM, has been shown to confer permanent donor-specific transplantation tolerance for subsequent skin, heart, and islet grafts in nonsensitized recipients (7–10). Due to the high incidence of allosensitization in the transplant population, the clinical application of mixed allogeneic chimeraism would be significantly limited if chimeraism and tolerance were precluded by the presence of preformed donor-reactive Abs or other anti-donor memory responses. In the current study, we have investigated the impact of allosensitization on BM engraftment and the induction of donor-specific tolerance. Although fully allogeneic reconstitution resulted in a high incidence of engraftment failure and death, stable allograftment and the induction of donor-specific transplantation tolerance were achieved in sensitized recipients utilizing a model of mixed allogeneic BM chimeraism. Survival, allograftment, and the induction of tolerance were significantly improved when large numbers (80 × 106) of allogeneic BM cells were administered several months after initial sensitization. Of even greater importance was the abrogation of allosensitization, demonstrated by the permanent loss of circulating anti-donor Abs and the induction of donor-specific tolerance following mixed allogeneic BMT.

Materials and Methods

Animals

Male, 6- to 8-wk-old C57BL/10SnJ (B10), B10.BR/SgSnJ (B10.BR), B10.D2, and BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were housed in a specific pathogen-free facility in the University of Pittsburgh Cancer Institute (Biomedical Science Tower, Pittsburgh, PA).

Skin grafting

Skin grafting was performed by a modification of the method of Billingham and Medawar (11). Full thickness skin grafts were harvested from the tails of B10 (H-2b), B10.BR (H-2k), BALB/c, or B10.D2 (H-2d). Full thickness graft beds were surgically prepared in the lateral thoracic wall of anesthetized mice, taking care to preserve the panniculus carnosus. Grafts were covered by a double layer of Vaseline gauze and a plaster cast to prevent shearing. A single donor graft was placed at the time of allosensitization. When assessing donor-specific tolerance, skin grafts from syngeneic, allogeneic donor, and third-party animals were placed on each recipient, leaving a 3-mm skin bridge separating each graft. Casts were...
removed on the seventh day, and grafts were scored daily for percent rejection. Reaction was considered complete when no residual viable graft could be seen. Graft survivals were calculated by the life-table method, and the median survival time (MST) was derived from the time point at which 50% of grafts were surviving (12).

Ab-dependent complement-mediated $^{51}$Cr microcytotoxicity assay

Serum was collected from recipient mice (B10 or B10.BR) before the placement of the sensitizing skin allograft (B10.BR/B10.D2 or B10, respectively) and weekly thereafter, with the exception of a 4-wk period immediately following BMT. Collected sera was stored at −20°C until needed for analysis, at which time it was decompartmented at 50°C for 30 min, serially diluted from 1/2 to 1/1024, and placed in 96-well round-bottom plates. A total of $2.5 \times 10^4$ $^{51}$Cr-labeled donor target splenocytes were added to each well, incubated at 37°C for 30 min, and washed at 1000 rpm. Following a second 30-min incubation at 37°C in the presence of rabbit complement (1/8), supernatants were collected and counted on a gamma counter (Titertek system, Skatron Instruments, Lier, Norway; a $^{51}$Cr gamma counter [Titertek system, Skatron Instruments, Lier, Norway; a $^{51}$Cr gamma counter].) Serum was collected from recipient mice (B10 or B10.BR) before the placement of the skin allograft and weekly during the rejection period, until allosensitization was documented for each animal. Serum was tested for the presence of donor-specific cytotoxic Abs against donor splenocytes, using a $^{51}$Cr microcytotoxicity assay. Anti-donor cytotoxic Abs were not present before (Fig. 1A, week 0) or at the onset of graft rejection (week 2). The rapid rise in anti-donor cytotoxic activity following allograft rejection (week 3) could be completely abrogated in vivo by the ip administration of cyclophosphamide. Cytotoxic Abs were donor specific, as evidenced by the absence of cytotoxicity toward third-party splenocytes, but not tissue specific, with marked cytotoxic activity against donor alloantigens on cells of either BM or splenic origin (Fig. 1B).

Results

Protocol for allosensitization: kinetics and specificity of Ab production

Donor-specific allosensitization was induced by placement of B10.BR or B10.D2 skin grafts on B10 recipients or B10 grafts on B10.BR recipients. Donor skin grafts were completely rejected by naive recipients with a MST of 12.4 days. Serum was obtained before placement of the skin allograft and weekly during the rejection period, until allosensitization was documented for each animal. Serum was tested for the presence of donor-specific cytotoxic Abs against donor splenocytes, using a $^{51}$Cr microcytotoxicity assay. Anti-donor cytotoxic Abs were not present before (Fig. 1A, week 0) or at the onset of graft rejection (week 2). The rapid rise in anti-donor cytotoxic activity following allograft rejection (week 3) could be completely abrogated in vivo by the ip administration of cyclophosphamide. Cytotoxic Abs were donor specific, as evidenced by the absence of cytotoxicity toward third-party splenocytes, but not tissue specific, with marked cytotoxic activity against donor alloantigens on cells of either BM or splenic origin (Fig. 1B).

Influence of donor BM composition on alloengraftment

The impact of allosensitization on conventional fully allogeneic BMT was demonstrated with the transplantation of increasing doses of allogeneic marrow in sensitized and nonsensitized recipients (B10.D2→B10, B10.BR→B10, and B10→B10.BR). Twelve weeks following sensitization, recipients were lethally irradiated and reconstituted with $15–80 \times 10^6$ allogeneic BM cells. Because the inoculum consists of only donor BM cells, long-term survival of fully allogeneic chimeras is indicative of donor engraftment. Survival of sensitized recipients increased in proportion to donor cell number, but remained markedly inferior to nonsensitized controls (Table I). Transplantation of $\leq 30 \times 10^6$ donor cells did not rescue recipients from aplasia, $40 \times 10^6$ cells resulted in a single 30-day survivor (9%), and $80 \times 10^6$ donor cells resulted in engraftment in only 25% of sensitized recipients.

To evaluate whether alloengraftment was improved under the auspices of syngeneic hematologic support, dose-titration studies were performed for mixed allogeneic reconstitution. Ten to thirteen weeks following sensitization, lethally irradiated recipients were reconstituted with a mixed inoculum of $5 \times 10^6$ T cell-depleted (TCD) syngeneic and $5–80 \times 10^6$ untreated allogeneic BM (B10 + B10.D2→B10; B10 + B10.BR→B10). Survival of mixed allogeneic chimeras was
100%, irrespective of the number of donor cells in the BM inoculum. Donor engraftment was assessed by the presence of donor lymphocytes in the peripheral blood (PBL) 1 mo following reconstitution. As evident in fully allogeneic reconstitution, alloengraftment in mixed chimeras was decreased in sensitized recipients and directly correlated with the size of the donor BM inoculum (Fig. 2). Donor engraftment was only achieved in those sensitized recipients reconstituted with BM inocula containing 60 × 10^6 or 80 × 10^6 allogeneic BM cells. Transplantation of mixed inocula (syngeneic plus allogeneic BM) significantly increased the incidence of donor engraftment with 73% (n = 8/11) of sensitized recipients reconstituted with 80 × 10^6 allogeneic BM cells exhibiting donor chimerism, as compared with 25% following fully allogeneic reconstitution. Once engraftment occurred, however, the level of donor chimerism was high, with mean chimerism 4–6 wk following mixed reconstitution with 60 × 10^6 or 80 × 10^6 allogeneic BM cells being 83.3 ± 16.7% and 98.8 ± 0.6%, respectively.

Several experimental and clinical studies have shown a negative effect on engraftment with T cell depletion of the donor BM inoculum using conventional TCD reagents, such as anti-Thy-1 Ab or RAMB antisera in murine systems (18–21). To determine whether TCD adversely affected alloengraftment in sensitized recipients, the incidence of donor chimerism was compared following mixed allogeneic reconstitution with RAMB-treated syngeneic and either untreated or RAMB-treated allogeneic BM. Despite the presence of 80 × 10^6 donor cells in each of the mixed inocula, RAMB treatment decreased the incidence of alloengraftment in sensitized recipients to 43% (n = 7), from 75% seen with untreated donor BM (n = 12).

FIGURE 1. Kinetics and specificity of cytotoxic Abs generated following skin allograft rejection. A, Sera from naive recipients were tested for the presence of anti-donor cytotoxic activity before placement of the donor skin graft (week 0) and at weekly intervals during graft rejection (six experiments, total n = 28). Serial dilutions (1/2–1/1024) of recipient sera were analyzed for each animal at each time point, and the maximum percentage of cytotoxicity ± SE was determined by analyzing ^51_Cr release from labeled donor splenocytes. Generation of donor-specific cytotoxicity was prevented by the administration of a single i.p. dose of cyclophosphamide (200 mg/kg) 2 days following placement of skin allografts (p values as shown for unpaired t test, n = 4). B, Sera from B10.BR recipients were tested for cytotoxic activity at 4 wk following placement of a B10 skin graft (n = 5/group). Serial dilutions of recipient sera were assayed for cytotoxic activity against the designated ^51_Cr-labeled BM or splenocyte targets. Cytotoxic activity was demonstrated against both BM and splenic tissues of B10 origin, but not against third-party BALB/c splenocytes.

FIGURE 2. Effect of BM dose on donor engraftment in allosensitized recipients. Lethally irradiated naive and allosensitized recipients were reconstituted with a mixture of 5 × 10^6 TCD syngeneic and 5 × 10^6 untreated allogeneic BM cells (B10 + B10.B2→B10; B10 + B10.BR→B10; B10.BR + B10→B10.BR). BMT was performed 10–12 wk following allosensitization, and chimeras were assessed for the presence of donor chimerism using flow-cytometric PBL typing 4–6 wk following reconstitution. The percentage of nonsensitized and allosensitized recipients exhibiting donor engraftment is shown for each donor inoculum.

Table 1. Influence of donor cell dose on engraftment in sensitized fully allogeneic chimeras

<table>
<thead>
<tr>
<th>No. of Cells in Donor BM Inoculum (×10^6)</th>
<th>Prior Skin Graft</th>
<th>n</th>
<th>% 30-Day Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Donor 5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>None 5</td>
<td>100</td>
<td>100</td>
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<tr>
<td>30</td>
<td>Donor 5</td>
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<td>Donor 11</td>
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<td>Donor 28</td>
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<td>100</td>
</tr>
<tr>
<td></td>
<td>None 16</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*Recipient mice were allosensitized with skin grafts 12 wks prior to BMT. Fully allogeneic chimeras (B10.D2→B10; B10.BR→B10, and B10→B10.BR) were prepared with increasing numbers of donor BM cells. Survival, indicative of donor engraftment, is compared at 30 days between naive and allosensitized recipients.*
Table II. Alloengraftment as a function of the anti-donor response of the recipient

<table>
<thead>
<tr>
<th>Anti-Donor Responsea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Prior to BMT</td>
</tr>
<tr>
<td>Prevented</td>
</tr>
<tr>
<td>Intact</td>
</tr>
<tr>
<td>Augmented</td>
</tr>
</tbody>
</table>

*a* B10 skin grafts were placed on B10.BR recipients 12 wk prior to reconstitution with a mixture of $5 \times 10^8$ TCD B10.BR and $60 - 80 \times 10^8$ untreated donor B10 BM cells (B10.BR + B10→B10.BR). The first group of recipients received 200 mg/kg cyclophosphamide i.p. 2 days following placement of the donor skin graft. The second group received an additional dose of 100 mg/kg prior to BMT. The third group of recipients received a second donor Ag challenge consisting of $60 \times 10^9$ donor splenocytes 2 days prior to mixed allogeneic BMT. Recipients were PBL typed for evidence of donor chimerism 4–6 wk following reconstitution.

Alloengraftment is dependent on initial recipient Ab response

The importance of the recipient immune status at the time of BMT on engraftment failure was demonstrated by assessing alloengraftment when recipient allosensitization was prevented or enhanced. Cyclophosphamide is a potent inhibitor of B cell-Ab responses, and inhibits conventional cellular immune responses at higher doses (22). Administration of this agent before donor Ag exposure thereby prevents the recipient from mounting humoral or cellular anti-donor immune responses. Despite the prior placement of a donor skin graft, alloengraftment readily occurred when allosensitization was prevented in vivo by the administration of cyclophosphamide (Table II). Conversely, alloengraftment was completely prevented when recipients, sensitized 12 wk earlier, were given a single i.v. bolus of $60 \times 10^9$ donor splenocytes as a second donor Ag challenge, 2 days before mixed allogeneic BMT.

Given these results, we hypothesized that a shorter interval between sensitization and BMT would also adversely affect alloengraftment. Five to seven weeks following sensitization, recipients underwent either fully or mixed allogeneic reconstitution. As predicted, BMT performed 5–7 wk following sensitization resulted in a higher rate of engraftment failure (Table III). This was most notably evident in fully allogeneic chimeras, as all recipients succumbed to aplasia. The incidence of donor chimerism following mixed reconstitution was decreased to 33%, but remained superior to fully allogeneic BMT. The decreased incidence of donor engraftment (33 vs 73%) evident in animals transplanted 5–7 wk following allosensitization corresponds to a higher Ab titer at the time of BMT (88 ± 8% maximum cytotoxicity, $n = 5$) as compared with animals presensitized 12 wk before BMT (42 ± 6% maximum cytotoxicity, $n = 18$, $p = 0.0001$).

Recipient Ab response and immunologic memory: maintenance of donor chimerism

Mixed allogeneic BMT was performed in 13 recipients, 5–7 wk following sensitization. Of these, 54% exhibited evidence of donor engraftment at 1 mo post-BMT. The correlation between failure of engraftment and sensitization suggested that recipients who failed to engraft must have exhibited high anti-donor Ab titers at the time of BMT. To test this hypothesis, the anti-donor cytotoxic activity just before mixed allogeneic BMT was compared between recipients with and without donor engraftment (Fig. 3). Surprisingly, anti-donor cytotoxic activity was not significantly different between these two populations at 64.3 ± 12.4% and 69.9 ± 11.2%, respectively (unpaired *t* test; $p = 0.625$).

The kinetics of cytotoxic activity, however, was significantly different, dividing recipients into two groups based on the degree and permanence of donor chimerism (Fig. 4). In the first group ($n = 4$), recipients exhibited high levels (>90%) of permanent donor chimerism that persisted throughout the 4-mo follow-up. These chimeras were characterized by a slow rise in anti-donor cytotoxic activity, reaching peak levels at the time of BMT (45.8 ± 10.7%). The second group exhibited low levels of donor chimerism (<5%), which disappeared by the fourth month, and thus donor marrow failed to engraft in these recipients. Recipients with transient or failed allografting ($n = 9$) were characterized by a rapid generation of high titer cytotoxic activity that began to slowly decline at the time of BMT (peak cytotoxicity = 88 ± 7.3%). The peak anti-donor cytotoxicity present in recipients with long-term donor engraftment never reached the high degree of cytotoxicity exhibited early in the course of sensitization by recipients that failed to engraft (unpaired *t* test; $p = 0.031$). Thus, despite similar anti-donor cytotoxic activity at the time of BMT (5–7 wk), the incidence of donor chimerism was quite different. These data indicate that anti-donor cytotoxic activity at the time of BMT is not...
the sole determinant of engraftment failure, but that engraftment might also be influenced by recipient immunologic memory mediated by cellular and/or humoral mechanisms.

Overcoming immunologic memory: donor-specific tolerance in sensitized recipients

Cellular immune responses were assessed in vitro and in vivo after mixed allogeneic reconstitution to determine whether recipients remained immunocompetent and if donor-specific tolerance had been achieved. In vitro proliferative MLR and cytotoxic CML assays revealed that lymphocytes from previously sensitized mixed allogeneic chimeras were functionally tolerant to both donor (B10) and host-strain (B10.BR) alloantigens (Fig. 5, A and B). Responses against third-party (BALB/c) alloantigens remained intact.

One month following BMT, a second donor skin graft was placed on previously sensitized mixed allogeneic chimeras, to assess the status of immunologic memory and donor-specific tolerance in vivo. There was an absolute correlation between stable allogeneic engraftment and tolerance. Despite the presence of allosensitization before BMT, recipients exhibiting donor chimerism continued to carry stable donor-specific Abs; 2) marrow is destroyed by a cellular immune response from the recipient alloimmune system; and 3) marrow is rejected by a humoral Ab response that is short-lived and fails to invoke a memory response, and the mortality following BMT in sensitized recipients was 100% (26, 28).

Loss of donor-specific Abs: re-education of recipient Ab response

If immunologic memory against donor Ags was truly altered following mixed reconstitution, one would expect circulating anti-donor Abs to be absent. As expected, high levels of donor-specific cytotoxicity persisted in recipients without long-term donor chimerism, 14 wk following BMT (73.5 ± 11.7%; n = 9). In contrast, recipients with stable chimerism had lost anti-donor cytotoxic activity (9.9 ± 7.4%; unpaired t test, p = 0.006). The absence of anti-donor Abs was maintained throughout the 4-mo follow-up period, providing evidence that immunologic memory responses can be altered as a result of successful mixed allogeneic reconstitution.

Discussion

Mixed allogeneic chimera has been shown to confer permanent donor-specific cellular transplantation tolerance for subsequent skin and solid organ allografts (7, 9, 23). Although mixed xenochimerism (mouse → rat → mouse) has been achieved in the presence of natural anti-rat BM Abs (7, 10, 24, 25), the impact of high titer alloantibodies on mixed allograftment and tolerance had not been previously investigated. Due to the high incidence of allosensitization in the transplant population, the clinical application of mixed chimerism would be significantly limited if allograftment and/or tolerance were precluded by the presence of preformed Abs or immunologic memory directed against the donor. Therefore, we were most interested in determining whether mixed chimerism could overcome the allosensitization barrier by either reducing or eliminating the generation of donor-specific Abs and by abrogating the cellular alloresponse that precludes BM engraftment and the induction of tolerance. The current report examines both donor and recipient factors preventing alloengraftment in sensitized recipients. It demonstrates that, unlike fully allogeneic models, mixed allogeneic reconstitution can occur in the presence of circulating anti-donor Abs, resulting in the establishment of allogeneic chimera, donor-specific transplantation tolerance, and the abrogation of the anti-donor Ab response.

Following rejection of skin allografts, anti-donor Abs prevented fully allogeneic reconstitution at all doses of donor BM less than 80 × 10^6. Even then, engraftment failure resulted in the death of 75% of all recipient animals. Several clinical and experimental studies of fully allogeneic BMT, including the initial reports by Barnes, Loutit, and Garver, have documented similarly high rates of engraftment failure in the presence of documented allosensitization (4, 26–30). Based on these findings, three explanations for engraftment failure in sensitized recipients have been proposed: 1) injected marrow is immediately targeted by circulating anti-donor Abs; 2) marrow is destroyed by a cellular immune response from sensitized radioreistant cells; or 3) both responses are involved. Demonstration that passive transfer of donor-specific antisera to nonsensitized recipients resulted in alloengraftment failure appeared to establish that anti-donor Abs alone were responsible (26, 28). Although subliminal levels of Ab were postulated to allow some donor marrow to survive, the contribution of a recipient cellular immune response to alloengraftment failure could not be assessed in these earlier models because passively transferred serum is short-lived and fails to invoke a memory response, and the mortality following BMT in actively immunized recipients was 100% (26, 31).

Death following fully allogeneic reconstitution is either the result of immediate rejection of the donor marrow, as suggested by Loutit et al. (26), or due to delayed or transient alloengraftment, whereby the recipient dies of aplasia despite the initial presence of viable donor marrow. In contrast, mixed allogeneic reconstitution provides hematologic support in the form of syngeneic BM that is neither delayed nor decreased by donor-specific Ab, thus assuring recipient survival and providing sufficient time for viable donor BM to engraft. This approach decreased engraftment failure 3-fold compared with fully allogeneic BMT. Evidence of donor chimerism at doses of 60 × 10^6 allogeneic cells suggests that some donor BM escapes the initial anti-donor cytotoxic response and remains viable in sensitized recipients. Although supported in a mixed allogeneic environment, surviving stem cells are insufficient to rescue fully allogeneic recipients. In addition to limited numbers of
viable allogeneic stem cells, TCD with RAMB antiserum has been demonstrated to concurrently remove facilitating marrow sub-populations (e.g., T cell subsets or facilitating cells), resulting in increased engraftment failure even in nonsensitized recipients (32). Facilitation of allogeneic stem cell engraftment appears to be equally important in sensitized recipients, as reconstitution with RAMB-treated donor BM decreased the incidence of donor chimerism by nearly half. The results of these studies demonstrate that by supporting recipient survival and permitting the delayed engraftment of allogeneic BM not immediately removed by circulating anti-donor Abs, mixed reconstitution provides the means to actively study alloengraftment in the sensitized recipient.

Although mixed allogeneic reconstitution with large doses of donor marrow resulted in alloengraftment in the majority of sensitized recipients, 25% either failed to initially exhibit donor chimerism or lost chimerism over the next few months. These failures highlight the importance of both: 1) the titer of circulating anti-donor cytotoxic Ab at BMT, and 2) the induction of an anti-donor memory response.

The incidence of donor chimerism was dramatically affected by differences in anti-donor cytotoxicity at the time of allogeneic BMT. Alloengraftment readily occurred when the initial anti-donor response was prevented with the administration of cyclophosphamide, but was significantly decreased in situations of high
anti-donor cytotoxicity, such as with a shortened transplant interval or second donor Ag challenge. However, anti-donor cytotoxic activity at the time of BMT was not the sole determinant of engraftment failure, demonstrating little difference between those recipients that went on to engraft with donor marrow and those that did not. The original claims that engraftment failure resulted from the presence of alloantibody alone were based on models in which recipient memory responses could not be assessed in situ (26, 31). Memory, or secondary responses, requires a long-term Ag-specific memory stem cell population to give rise to shorter-lived progeny each time Ag is encountered (33). Several studies have shown that the magnitude of a memory response is a function of the number of immune cells recruited during the primary response (34–36). The importance of this fact is illustrated in the current study by the higher and earlier peak in anti-donor cytotoxic activity that is evident in sensitized recipients that subsequently fail to engraft. Thus, despite similar Ab titers at the time of BMT, recipients with the greatest premium of Ab activity at the initial response, and thus greater Ab production during a secondary memory response, exhibit increased rates of engraftment failure.

Immunologic memory decays in a biphasic fashion (34–36). The initial decline over the first 40 days is rapid, reflecting the loss of Ag-specific progeny, or Ab-producing cells. The later phase is akin to the senescence of unstimulated memory cells and has a $a_{1/2}$ of ~100–200 days (34–37). The marked difference in successful allograftment between recipients transplanted at 5 or $\geq$12 wk following sensitization parallels these two phases of immunologic memory. Due to the presence of large numbers of activated memory and Ab-forming cells, peak Ab titers are significantly greater if Ag restimulation occurs during the early phase (34). This explanation may account for the increase in engraftment failure noted in recipients transplanted early after sensitization, despite the fact that circulating Ab titers were similar in recipients transplanted later. When restimulation occurs during the secondary phase, as when BMT is delayed, the secondary Ab response is decreased because Ab-forming cells must first be derived from the remaining memory cells before producing Ab. The delay in BMT did allow the peripheral Ab response to decay, but it did not disappear completely and immunologic memory was not lost before BMT. This was evident in the poor survival following delayed fully allogeneic reconstitution and the absence of allograftment following a second donor Ag challenge given immediately before BMT. Therefore, although high donor cell numbers and decaying Ab titers may permit the initial engraftment of viable donor marrow to occur in the sensitized host, under the guise of syngeneic hematologic support, the induction of multilineage chimerism and donor-specific tolerance must re-educate the recipient immunologic memory to promote the maintenance of long-term allograftment.

Stable donor chimerism following mixed allogeneic reconstitution was uniquely characterized by the loss of anti-donor Abs and the induction of donor-specific transplantation tolerance assessed in vivo and in vitro. The resurgence of anti-donor cytotoxic activity in lethally irradiated recipients that failed to engraft with donor marrow lends support to the hypothesis that memory must be maintained within a radioreistant recipient population. The follicular dendritic cell has been postulated to be the in vivo source of antigenic stimulation necessary for the maintenance of memory, and interestingly, also the induction of tolerance (38–41). Positive and negative selection pathways, documented to be functional following mixed reconstitution, may prove to be critical to the success of mixed allogeneic BMT in sensitized recipients (8, 42–44).

In the current study, mixed allogeneic reconstitution achieved allograftment even early after allo sensitization in a significant number of recipients. This feat was not possible, despite lethal conditioning, with fully allogeneic BMT. Although it is possible that mixed chimerism may merely provide syngeneic support for subsequent allogeneic engraftment in the sensitized recipient rather than a true reversal of the sensitized state, it is important to recognize that some of the sensitized recipients, despite the presence of syngeneic BMT and transient allograftment, failed to exhibit long-term engraftment of the allogeneic BM component, donor-specific tolerance, or loss of circulating anti-donor Abs. This observation suggests that the mere presence of syngeneic support is not in and of itself sufficient to permit allogeneic engraftment in a sensitized recipient. It also supports the hypothesis that the sensitized state must be abrogated to permit a stable state of allograftment. Although the conditioning regimen itself, and not the state of mixed chimerism per se, may be hypothesized to blunt or eliminate the memory response, it must be noted that only those animals that achieve mixed allogeneic chimerism concurrently attain a state of donor-specific tolerance. Recipients that underwent an identical conditioning and transplant regimen, but fail to demonstrate mixed allogeneic chimerism, maintain an alloresponse and reject donor skin grafts. Unlike earlier conclusions, based on models of fully allogeneic reconstitution, these results suggest that the ability to achieve donor BM engraftment in a sensitized host is determined by both the circulating Ab titer at BMT and the status of the recipient immunologic memory response. We anticipate that success may be further enhanced in those recipients with extremely vigorous anti-donor responses by combining the therapies of immunosuppression, immunosuppression, and mixed reconstitution. It is our hope that the clinical application of mixed allogeneic chimerism will eventually result in a drug-free state of donor-specific transplantation tolerance and recipient immunocompetence that will extend the application of BM and solid organ transplantation to include sensitized recipients currently denied this potentially life-saving therapy.

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References
ABROGATION OF ALLOSENSITIZATION WITH MIXED ALLOGENEIC CHIMERISM

induce donor-specific tolerance to sequential or simultaneous islet xenografts. 


