Induction of Species-Specific Host Accommodation in the Hamster-to-Rat Xenotransplantation Model

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The combination of two immunosuppressants, leflunomide and cyclosporin A (CsA), completely inhibits immune xenoreactions in the hamster-to-Lewis rat xenotransplantation model. In addition, the control of acute xenograft rejection with this combination of immunosuppressants subdues early T-independent xenoreactivity and uncovers a late immune response that can be controlled by CsA alone. We attribute this acquired responsiveness to CsA to a modification in the recipient’s humoral response to the xenograft, and refer to this change as host accommodation. Host accommodation can be induced in Lewis rats receiving hamster hearts by the combination of leflunomide and CsA. A 7-day treatment with leflunomide and CsA was able to convert xenoreactivity from one that was resistant to CsA treatment into one that was controlled by CsA. The presence of the hamster xenograft was critical for the induction of host accommodation since the immunosuppressive regimen, either alone or in combination with a transfusion with donor-specific spleen cells, was unable to modify the anti-hamster reactivity in Lewis rats. When accommodation was induced in the presence of hamster hearts, these accommodated rats were able to acutely reject third-party mouse hearts while under CsA therapy, thus indicating that the host accommodation is species specific. Finally, we demonstrate that host accommodation is associated with a loss in the ability to produce species-specific, T-independent xenoaebodies. These novel observations suggest that xenoreactive T-independent humoral responses can be deleted selectively without significant loss of other innate, Ag-specific T-independent humoral responses. The Journal of Immunology, 1998, 161: 2044–2051.

R

everse success in controlling complement-mediated hyperacute rejection has shifted the focus of xenotransplantation from the control of hyperacute rejection to the control of acute vascular rejection (AVR) or delayed xenograft rejection (DXR) (1–3). There is increasing experimental evidence that xenoaebodies (XAbs) are important mediators of AVR/DXR and that the production of XAbs during AVR/DXR in the pig-to-primate model is remarkably resistant to conventional immunosuppression (4). XAb production can be controlled significantly by triple immunosuppression with cyclophosphamide, cyclosporine, and steroids, leading to extended xenograft survival (1, 5). However, severe bone marrow toxicity limits the extended use of cyclophosphamide (6, 7). Thus, there is an urgent need for alternative approaches to controlling the elicited XAb responses seen in transplantations of pig-to-baboon and, presumably pig-to-human.

The humoral response to xenografts in the concordant model of hamster-to-Lewis rat xenograft transplantation is T cell independent and resistant to conventional T cell-specific immunosuppressive agents. Effective control of xenograft rejection has been achieved with combinations of antiproliferative and immunosuppressive agents (8–10). These observations were obtained initially by Hasan and his colleagues using the combination of cyclophosphamide and cyclosporine (11–14). In an extensive study by Murase et al. (15), stable long-term survivals of hamster heart and liver xenografts were achieved successfully by combining six different antiproliferative drugs, including cyclophosphamide, with the immunosuppressant FK506. Most impressive were their observations that a transient treatment with the antiproliferative agents resulted in long-term xenograft survival as long as FK506 treatment was maintained.

We and others have reported that the novel immunosuppressant, leflunomide, in combination with CsA or FK506, completely prevents the rejection of hamster xenografts in rats (16–18). Cessation of all immunosuppression invariably results in xenograft loss. We referred to this rejection initially as DXR (19) but, more recently, as late xenograft rejection (LXR) to avoid confusion with the rejection of discordant xenografts after complement is inactivated, which is also labeled DXR (19). Histologic examination of the grafts undergoing LXR indicates the presence of large numbers of macrophages, deposited IgM, edema, hemorrhage, and myocyte necrosis. Thus, while LXR is observed in a discordant transplantation model, it has many histologic features of AVR/DXR, as described in discordant systems.

In contrast to acute xenograft rejection of hamster hearts by Lewis rats, LXR can be controlled completely by CsA monotherapy (19, 20). We interpret these observations as indicating that the mechanism of LXR is different from that of acute xenograft rejection. Recent studies suggest that a modification of the immune response in the Lewis rat to the hamster xenografts was responsible for this acquired sensitivity to CsA. First, the rejection of a second hamster heart transplanted into an accommodated, but not in a normal, Lewis rat could be controlled by CsA alone (21). Second, the production of XAbs was inhibited by CsA in accommodated Lewis rats, but not in normal freshly transplanted Lewis rats (22–24). Third, the XAbs produced during acute xenograft
Both leflunomide and CsA are required for the induction of host accommodation

<table>
<thead>
<tr>
<th>First Treatment</th>
<th>Second Treatment (day 14 to rejection)</th>
<th>First Heart Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lef (10 mg/kg/day)</td>
<td>CsA (20 mg/kg/day)</td>
<td>19, 19, 19</td>
</tr>
<tr>
<td>Lef (15 mg/kg/day)</td>
<td>CsA (20 mg/kg/day)</td>
<td>19, 19, 21</td>
</tr>
<tr>
<td>Lef (20 mg/kg/day)</td>
<td>CsA (20 mg/kg/day)</td>
<td>19, 19, 19</td>
</tr>
<tr>
<td>Lef + CsA</td>
<td>CsA (20 mg/kg/day)</td>
<td>&gt;84, &gt;84, &gt;86, &gt;77, &gt;77</td>
</tr>
<tr>
<td>Lef + CsA</td>
<td>Lef (15 mg/kg/day)</td>
<td>&gt;24, &gt;24, &gt;56</td>
</tr>
<tr>
<td>CsA (20 mg/kg/day)</td>
<td>None</td>
<td>4, 4, 4, 5, 15</td>
</tr>
</tbody>
</table>

* For the Lef + CsA group, the doses were leflunomide (Lef; 5 mg/kg/day) and CsA (20 mg/kg/day). Both immunosuppressive agents were administered orally.
* Lewis recipients were treated for 21 days with Lef (5 mg/kg/day) + CsA (20 mg/kg/day), and subsequently with leflunomide monotherapy (15 mg/kg/day). Lewis rats were sacrificed on the indicated days due to severe anemia with weakly beating xenograft hearts.

rejection, but not during LXR, were able to induce the hyperacute rejection of freshly transplanted hamster hearts in naive Lewis rats (21). We have referred to this modification of the host immune reaction as host accommodation, and hypothesize that this modification reflects an inactivation or tolerance of the T-independent xenoreactive antibody response. In this work, we describe a series of experiments that focus on further defining the conditions required for the induction of host accommodation in this rodent model of xenotransplantation, and the mechanism of host accommodation.

Materials and Methods

Transplantation model

Lewis rats, BALB/c mice, and Golden Syrian hamsters were purchased from Harlan Labs (Indianapolis, IN). In some transplants, C57BL/6 SJL/JF1 mice that were used were a gift from Dr. Arjay Sharma (Nextran, Princeton, NJ). Hamster or mouse hearts were heterotopically transplanted into the abdomen or the right groin of the recipients following a modified protocol described by Ono et al. (25). In some experiments, a third transplant, from Harlan Labs (Indianapolis, IN). In some transplants, (C57BL6/SJL)F1 mice that were used were a gift from Dr. Arjay Sharma (Nextran, Princet-

Immunosuppression

Cyclosporine (20 mg/kg/day) in oral form (Sandimmune, Sandoz, East Hanover, NJ) was suspended by sonication in water, while leflunomide (5–20 mg/kg/day; custom synthesized for research purposes) was suspended in 1% carboxymethyl cellulose. Both drugs were administered by gavage.

Histology and immunohistochemistry

All heart grafts were harvested, embedded in OCT, and immediately snap frozen in liquid nitrogen. The hearts were sectioned (5 μm) and stained with hematoxylin and eosin (H&E). Other sections for immunohistochemical staining were subject to the standard avidin-biotin conjugate (ABC) method. Briefly, microsections were fixed with cold acetone, then incubated serially with 0.015% H2O2, 5% goat serum, anti-rat IgM or anti-rat IgG (MARM-4 or MARG-2; Serotec USA, Washington DC), biotinylated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA), and then with hors eradish peroxidase-conjugated streptavidin (Zymed Labs, South San Francisco, CA). Chromogen, 3,3'-diaminobenzidine solution was added, and the slides were counterstained with Mayer’s hematoxylin. For all histologic sections, isotype-matched controls of purified rabbit or goat Ig were performed in parallel.

Quantification of hamster-specific IgM and IgG Abs

Quantification of hamster-specific Abs was performed as previously described (16, 19). A total of 5 × 107 golden Syrian hamster and BALB/c mouse erythrocytes was incubated with diluted heat-inactivated test serum or control naive Lewis rat serum (1/20 dilution) for 30 min at 4°C. Erythrocytes were washed in 4% (w/v) sodium citrate/PBS, then stained with phycoerythrin-conjugated F(ab)2 anti-rat IgM or FTTC-conjugated F(ab)2 anti-rat IgG (Jackson ImmunoResearch). After staining, the erythrocytes were washed, fixed in 1% Formalin, and analyzed using a flow cytometer (Ortho Cytoron Absolut, Ortho Diagnostic Systems, Raritan, NJ).

Results

Leflunomide and CsA are both required for the induction of host accommodation

It has been established that a transient treatment with leflunomide and CsA induces host accommodation in the hamster-to-rat xenotransplantation model (17, 21, 26) (Table I). In contrast, hamster hearts are rejected in 4 to 15 days when treated with CsA (20 mg/kg/day) alone (21) (Table I), and in 7.7, 52, 77, and 59 days when treated with leflunomide alone at doses of 5, 10, 15, and 20 mg/kg/day, respectively (16). We first asked whether host accommodation can be induced with leflunomide monotherapy, or whether it required the combination of leflunomide and CsA. Lewis rats receiving a hamster heart were treated with leflunomide, at doses of 10, 15, and 20 mg/kg/day, for 14 days, then maintained on CsA (20 mg/kg/day). While immunosuppression with leflunomide monotherapy (10–20 mg/kg/day) resulted in the complete inhibition of xenoreactive IgM production, host accommodation was not induced and the hamster hearts were rejected in 5 to 6 days after immunosuppression was switched to CsA monotherapy (Table I). Xenograft rejection was associated with a 5.6-fold increase in the levels of hamster-reactive IgM (Fig. 1b). These elevated levels of xenoreactive IgM were comparable with those observed...
The next series of experiments were designed to determine the minimum time of immunosuppression required for the induction of host accommodation. It has been reported previously that a 21-day treatment with leflunomide and CsA allowed subsequent hamster heart survival to be maintained on CsA alone (17, 21, 26). We demonstrate in this study that the minimum time of combination treatment with leflunomide and CsA that allowed hamster hearts to be maintained on CsA alone was 7 days (Table II). Extended survival of the hamster hearts corresponded with effective inhibition of the XAb production by CsA after either a 7- or 14-day treatment with leflunomide and CsA (Fig. 1c). In contrast, Lewis recipients of hamster hearts that were treated with 20 mg/kg/day of CsA alone produced hamster-reactive IgM by 4 to 16 days posttransplantation (Fig. 1a). These results confirm and extend previous observations that a transient treatment with the combination of leflunomide and CsA effectively subdues the T-independent XAb response and uncovers a T-dependent response that is controllable by CsA alone (21–24).

In contrast to CsA, leflunomide (15 mg/kg/day) was unable to maintain long-term survival of the hamster grafts in accommodated Lewis rats (Table I). Following 21 days of combined treatment with leflunomide (5 mg/kg/day) and CsA (20 mg/kg/day), an immunosuppressive dose of 15 mg/kg/day of leflunomide resulted in severe anemia by 24 or 56 days posttransplantation, and the Lewis recipients were sacrificed. At this time, the xenograft hearts were beating weakly. Histologic examination revealed signs of intimal thickening and mononuclear infiltration, similar to hamster hearts maintained on leflunomide monotherapy for the duration of the experiment (data not shown) (16). These observations are consistent with those previously reported by Lin et al. (27) in a hamster into PVG rat transplantation model, and indicate that CsA and control of T cell xenoreactivity are critical for the maintenance of long-term graft survival.

**Induction of host accommodation requires the presence of the hamster heart grafts**

It is possible that potent immunosuppression with leflunomide and CsA resulted in a nonspecific loss in the immune reactivity, and an apparent induction of host accommodation. We reasoned that if this were the case, then host accommodation should be induced in the absence of the xenograft. We therefore investigated whether treatment with leflunomide and CsA in the absence of a xenograft would result in a subsequent inability of Lewis rats to reject hamster hearts while under CsA alone. As presented in Table III, Lewis rats pretreated with leflunomide and CsA for 14 days, before transplantation with a hamster heart, were able to reject hamster hearts in 4 to 7 days while on CsA monotherapy.

Further evidence of the importance of Ag in the induction of host accommodation came from the next series of experiments. It has been reported that donor-specific transfusion with spleen cells.

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**Table II. Time course of combination treatment**

<table>
<thead>
<tr>
<th>First Treatment</th>
<th>Duration</th>
<th>Second Treatment</th>
<th>First Heart Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lef + CsA</td>
<td>21 days</td>
<td>CsA &gt; 21 days</td>
<td>&gt;83, &gt;83, &gt;90, &gt;81</td>
</tr>
<tr>
<td>Lef + CsA*</td>
<td>14 days</td>
<td>CsA &gt; 14 days</td>
<td>&gt;84, &gt;84, &gt;86, &gt;77, &gt;77</td>
</tr>
<tr>
<td>Lef + CsA</td>
<td>7 days</td>
<td>CsA &gt; 7 days</td>
<td>&gt;67, &gt;72, &gt;98, &gt;91</td>
</tr>
</tbody>
</table>

* Data of the five Lewis recipients were treated for 14 days with leflunomide (Lef, 5 mg/kg/day) + CsA (20 mg/kg/day) are the same as presented in Table I.

**Table III. Role of the xenograft in the induction of host accommodation**

<table>
<thead>
<tr>
<th>First Treatment (day 0–14)</th>
<th>Second Treatment (day 14 to rejection)</th>
<th>Day of Heart Transplant</th>
<th>Heart Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lef + CsA</td>
<td>CsA</td>
<td>0</td>
<td>&gt;84, &gt;84, &gt;86, &gt;77, &gt;77</td>
</tr>
<tr>
<td>Lef + CsA</td>
<td>CsA</td>
<td>14</td>
<td>18, 18, 20, 21</td>
</tr>
<tr>
<td>Lef + CsA + splenocytes</td>
<td>CsA</td>
<td>14</td>
<td>18, 18, 20, 24</td>
</tr>
</tbody>
</table>

* Splenocytes (50 × 10⁵/Lewis recipient) from hamsters were administered intravenously on day 0. Immunosuppressive agents, leflunomide (Lef; 5 mg/kg/day) and/or CsA (20 mg/kg/day), were administered orally.

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**Table IV. Host accommodation is species-specific**

<table>
<thead>
<tr>
<th>Day of Transplant</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Hamster Heart</td>
<td>0</td>
</tr>
<tr>
<td>Second Hamster Heart</td>
<td>14–27</td>
</tr>
<tr>
<td>First Mouse Heart</td>
<td>74–82</td>
</tr>
<tr>
<td>First Mouse Heart</td>
<td>0</td>
</tr>
<tr>
<td>Second Mouse Heart</td>
<td>14–27</td>
</tr>
<tr>
<td>First Hamster Heart</td>
<td>60–72</td>
</tr>
<tr>
<td>Transplant into naive Lewis Rats</td>
<td>Mouse</td>
</tr>
<tr>
<td>Mouse (CsA 20 mg/kg/day; day 0 to rejection)</td>
<td>0</td>
</tr>
<tr>
<td>Hamster</td>
<td>0</td>
</tr>
<tr>
<td>Hamster (CsA 20 mg/kg/day; day 0 to rejection)</td>
<td>0</td>
</tr>
</tbody>
</table>

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* Accommodation was induced in Lewis rats with a hamster or mouse cardiac graft and treated with leflunomide (5 mg/kg/day) + CsA (20 mg/kg/day) for 14 days. Accommodated Lewis rats were maintained on CsA until rejection of third species heart. Survival is calculated from the day of transplantation.

* Data of the five Lewis recipients were treated for 14 days with leflunomide (5 mg/kg/day) + CsA (20 mg/kg/day) are the same as presented in Table I.
synergizes with a variety of immunosuppressive protocols to induce tolerance to allografts in mice, rats, and humans (28–31). Lin et al. reported that immunization of rats with hamster spleen cells (20 \times 10^6/mouse) under leflunomide monotherapy (20 mg/kg/day) could induce T-independent accommodation to a second challenge of hamster splenocytes (32). We therefore tested whether hamster spleen cells could induce host accommodation and allow late xenoreactivity to hamster hearts to be subdued by CsA alone. Hamster spleen cells (50 \times 10^6/Lewis rat) were administered i.v. on day 0 into Lewis rats treated with leflunomide and CsA for 14 days.

FIGURE 2. Lewis rats accommodated to hamster hearts are able to reject a third-party mouse heart. Lewis rats were transplanted with hamster hearts on days 0 and 14 to 27, and mouse hearts were transplanted on days 74 to 82. These Lewis rats were treated with leflunomide (Lef) for 14 days, and CsA (20 mg/kg/day) for the duration of the experiment. Hearts were harvested on the day of rejection of the mouse heart. A–C and D–F are sections from the first and second hamster hearts, respectively, and G–I are sections from the third-party mouse hearts. A, D, and G are hematoxylin and eosin (H&E)-stained sections; B, E, and H are immunohistochemical demonstrations of deposited IgM; and C, F, and I are ED-1 mAb stainings of tissue macrophages.
days. On day 14 posttransplantation, immunosuppression was switched to CsA monotherapy and the Lewis rats were transplanted with a hamster heart (Table III). All hamster hearts were rejected in 4 to 10 days, indicating that donor-specific transfusion of hamster spleen cells could not substitute for a hamster heart to induce host accommodation to hamster hearts, and that host accommodation is at least partially tissue specific.

Specific induction of host accommodation to hamster hearts

The importance of xenograft, and the inability of hamster spleen cells to induce host accommodation to hamster hearts led us to hypothesize that this process could also be species specific. Hamster hearts were transplanted into Lewis rats treated for 14 days and CsA (20 mg/kg/day) for the duration of the experiment, and transplants were performed on the days indicated by the arrows. Sera were harvested on the indicated days posttransplantation. All data are presented as mean channel fluorescence (MCF) and represent the means of three to six individual animals per group (±SE).

Specific induction of host accommodation to mouse hearts

The observation that Lewis rats can be specifically accommodated to hamster hearts, yet retain the ability to reject mouse hearts while under CsA therapy, is a novel and potentially significant one. To extend this observation and to further characterize the specificity of the accommodation process, we performed the converse experiment. We tested whether Lewis rats could be specifically accommodated to mouse hearts, yet retain the ability to reject hamster hearts.
Specificity of xenoreactive Abs during the acute rejection of hamster hearts: challenge with mouse heart transplants

It has been reported previously that the XAbs produced by Lewis rats acutely rejecting hamster hearts are cross-reactive to mouse Ags (33, 34). We have extended these findings and have characterized the cross-reactivity of the Abs produced during acute rejection. Pooled serum from rats with acutely rejected hamster hearts while under CsA. Presented in Table IV are data indicating that specific accommodation could also be induced to mouse hearts. In addition, the data suggest that accommodation in this combination is also species specific, as five of six Lewis rats accommodated to mouse heart were able to reject the hamster heart while retaining the accommodated mouse heart (Table IV).

We also quantified the levels of anti-hamster and anti-mouse IgM in these accommodated rats. Five of the six Lewis rats accommodated to mouse hearts were unable to produce mouse-reactive Abs while under CsA monotherapy, but were able to reject a third-party hamster heart. The rejection of the hamster heart was accompanied by a 6.3-fold increase in the levels of anti-hamster IgM, while no detectable increase observed in the levels of cross-reactive anti-mouse IgM during the acute rejection of hamster hearts by Lewis rats (Fig. 3a). At the time of acute rejection of hamster hearts in untreated or CsA-treated Lewis rats, the levels of anti-mouse IgM were elevated 11.3- and 8.6-fold, respectively (Fig. 3c). The levels of cross-reactive anti-hamster IgM in untreated or CsA-treated Lewis rats were elevated 4.8- and 4.7-fold, respectively (Fig. 3c). These observations collectively support the notion that host accommodation involves a species-specific inactivation of T-independent, xenograft-specific B cell responses in Lewis rats.

Discussion

Previous histologic analyses of the spleen of Lewis rats upon transplantation of a hamster heart suggested that both T-independent and T-dependent responses were involved in the process of acute xenograft rejection (35). It is possible to suppress the T-dependent response with agents that block T cell activation, and to demonstrate that the remaining T-independent response is able to acutely reject hamster xenografts. However, it has not been possible to suppress only the T-independent response; thus, T-dependent xenoreactive responses have been poorly characterized to date. The studies presented in this work, as well as recent published studies (20, 21, 23, 26), suggest that a transient treatment with leflunomide and CsA eliminates the T-independent, but not the T-dependent response
response. Thus, this model system provides a unique opportunity to investigate T-dependent xenograft-specific responses.

It has been reported previously that the XAbs produced in the rat during the acute rejection of the hamster heart xenografts also cross-react with and mediate the hyperacute rejection of mouse cardiac grafts (33, 34). We have extended those findings and report in this work that T-independent XAbs, produced during the acute rejection of hamster grafts in the presence of CsA, can also mediate the hyperacute rejection of mouse hearts. This observation suggests that the repertoire of T-independent XAbs produced during the acute rejection of a hamster heart can be divided into those that are hamster specific and those that cross-react with hamster and mouse Ags.

It has been reported that a transient treatment with leflunomide and CsA, after transplantation of a hamster heart, converts the anti-hamster response from one that is CsA resistant to one that is controlled completely by CsA alone. We and others have concluded that treatment with leflunomide and CsA eliminates the host’s T-independent response, but retains the T-dependent response that is controllable by CsA monotherapy (20, 21, 23, 26). Earlier published studies indicate that this T-dependent anti-hamster response is distinct from the T-independent response, and demonstrate that the T-independent XAbs have reduced ability to induce C3 deposition and hyperacute rejection of hamster hearts (21). We have referred to this change in xenograft-specific response as host accommodation.

We demonstrate in this study that both CsA and leflunomide are required for the induction of host accommodation, as is the presence of the xenograft. Furthermore, hamster spleen cells were unable to induce host accommodation to hamster hearts. Recent studies by Lin et al. reported that immunizing with hamster spleen cells in the presence of leflunomide induced T-independent B cell tolerance and the inability to respond to a second challenge of hamster spleen cells (32). Their observations along with ours collectively suggest that host accommodation is at least partially tissue specific.

The importance of the appropriate Ag in the induction of accommodation to hamster hearts is further illustrated by the ability of these animals to reject a third-party mouse heart. Conversely, Lewis rats accommodated to mouse hearts were able to reject hamster hearts. Collectively, these novel observations suggest that the induction of host accommodation is both species specific and tissue specific. These observations complement and extend observations reported recently by Lin et al. (36) of the induction of specific tolerance across xenogeneic barriers in the hamster heart into nude rat transplantation model. They demonstrated that host accommodation in their model was based on species-specific NK cell tolerance and a non-species-specific tolerance of the T-independent xenoreactive response.

The specificity of the host accommodation in this xenotransplantation model is especially striking because sensitization to hamster hearts also sensitizes the recipient to mouse hearts. In fact, both the T-dependent and the T-independent Abs produced during the rejection of a hamster heart cross-react with mouse Ags. To explain the Ab specificity observed during the different rejection events, we have diagrammed in Figure 5 the clones of B cells that are stimulated by a hamster xenograft. In naive Lewis rats, the hamster graft stimulates both hamster-specific and hamster-mouse cross-reactive B clones, and both T-independent and T-dependent clones are stimulated. CsA is able to inhibit the activation and expansion of only the T-dependent B cell clones (Fig. 5a). Host accommodation in the presence of a hamster graft results in the elimination or inactivation of T-independent B cell clones that are either hamster specific or hamster-mouse cross-reactive (Fig. 5b). The T-independent B cell clones that are mouse specific are not affected by the accommodation process, and can subsequently mediate the rejection of a freshly transplanted mouse heart in the presence of CsA therapy (Fig. 5b). The T-dependent, anti-hamster
or anti-mouse B cells that are not tolerized are immunesuppressed with continuous CsA monotherapy (Fig. 5b). Finally, when CsA treatment is stopped, the accommodated Lewis rats reject the hamster heart by a process termed LXR. T-dependent B cells that are either hamster specific or hamster-mouse cross-reactive become activated to produce Abs during LXR.

In conclusion, we have made the novel observation that host accommodation can be induced in Lewis rats receiving hamster hearts by the combination of leflunomide and CsA, but not with either of these agents alone. The presence of the hamster xenograft was critical for the induction of host accommodation since the immunesuppressive regimen, alone or in combination with donor-specific transfusion of spleen cells, was unable to modify the anti-hamster reactivity in Lewis rats. We also report that when accommodation was induced in the presence of hamster hearts, these Lewis rats were able to acutely reject third-party mouse hearts while under CsA therapy. Finally, host accommodation in Lewis recipients of hamster hearts is associated with the loss in the ability to produce T-independent, hamster-reactive Abs. This novel observation that host accommodation is Ag specific suggests that xenoreactive T-independent humoral responses can be selectively deleted without significant loss of other innate Ag-specific T-independent humoral responses.

Acknowledgments

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References