SUPPRESSION OF ALLOGRAFT REJECTION WITH FK506

I. PROLONGED CARDIAC AND LIVER SURVIVAL IN RATS FOLLOWING SHORT-COURSE THERAPY

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Heterotopic heart and orthotopic liver grafts from ACI donors were transplanted to Lewis rat recipients that were treated with a 3 (or 4) day course of FK506 (originally FR90056) dissolved in saline and administered once a day by intramuscular injection. Treatment can induce prolonged cardiac allograft survival in rats (5) and liver or kidney survival in dogs (11, 12). In the present study, we have examined in more detail the effect of a 3-4-day course of FK506 treatment on the subsequent survival of heart and liver allografts in rats. In a companion study (18), we have characterized the changes of the host immune response that result from this therapeutic approach.

MATERIALS AND METHODS

**Animals.** Inbred male rats were used in all experiments. Lewis rats (LEW) weighing 250-350 g (Harlan Sprague-Dawley Inc., Indianapolis, IN) were the recipients, and ACI rats weighing 150-250 g (Simonsen Laboratories, Gilroy, CA) were donors. All animals were maintained in conventional animal facilities with water and commercial rat chow provided ad libitum.

**FK506 administration.** FK506 (originally E5258) was discovered in 1984 (1,2) and has become an object of considerable interest in the field of organ transplantation. Prolongation of survival times has been demonstrated for skin, heart, kidney, or liver allografts, in rodents (3-7), subhuman primates (11,12), and humans (13). FK506 is more potent than cyclosporine and has a similar mode of action, including inhibition of T lymphocyte response to stimulation with mitogen, or stimulation with alloantigens in mixed lymphocyte reactions (14,15). It inhibits the induction of allo specific human and mouse cytolytic T lymphocytes, and it suppresses IL-2 and gamma-IFN secretion and IL-2 receptor expression of the T lymphocytes (2,14-17).

The novel immunosuppressive agent FK506 (originally FR90056) was discovered in 1984 (1,2) and has become an object of considerable interest in the field of organ transplantation. Prolongation of survival times has been demonstrated for skin, heart, kidney, or liver allografts, in rodents (3-7), subhuman primates (11,12), and humans (13). FK506 is more potent than cyclosporine and has a similar mode of action, including inhibition of T lymphocyte response to stimulation with mitogen, or stimulation with alloantigens in mixed lymphocyte reactions (14,15). It inhibits the induction of allo specific human and mouse cytolytic T lymphocytes, and it suppresses IL-2 and gamma-IFN secretion and IL-2 receptor expression of the T lymphocytes (2,14-17).

What FK506 does not do is of equal interest. It does not at any concentration inhibit the B-cell-stimulating factor 2 driven proliferation of cloned B cells, nor does it inhibit IL-2-driven proliferation of cloned T cells (17). Thus, FK506 has been said to inhibit the proliferation of lymphocytes during the recognition and induction phase of the allograft reaction and not the proliferation seen subsequent to the production and binding of lymphokines. If this were its complete profile, the effectiveness of FK506 in preventing allograft rejection would depend upon administration of the drug very early in the posttransplantation period.

We have previously shown that a brief course of FK506 treatment can induce prolonged cardiac allograft survival in rats (5) and liver or kidney survival in dogs (11, 12). In the present study, we have examined in more detail the effect of a 3- or 4-day course of FK506 treatment on the subsequent survival of heart and liver allografts in rats. In a companion study (18), we have characterized the changes of the host immune response that result from this therapeutic approach.

**RESULTS**

Survival in cardiac allografts. Untreated LEW recipients rejected ACI heart grafts with median graft survival time of 6 days (group 1, Table 1). As we previously reported in a study on FK506 dose-response relations (5), treatment with daily intramuscular FK506 (1.28 mg/kg) for the first 2 weeks after grafting significantly prolonged graft survival (group 2, MST:

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time for the group, but it did reduce the time range in which rejection occurred (Table I). Increasing the amount of drug administered (2.5 mg/kg/day; group 8) did not prolong the median graft survival length of treatment (days 3, 4, 5, 6; group 7) or increasing the amount of drug administered (2.5 mg/kg) (group 3) did not prolong the median graft survival time. FK506 treatment early in the posttransplantation period markedly reduced the number of inflammatory cells. The allograft reaction in the heart consists of a diffuse, rapidly progressive mononuclear infiltration of the myocardium. By day 6, the inflammatory reaction had caused sufficient damage to the myocardium so that the graft ceased to function (Fig. 1A). In contrast, the hearts in FK506-treated animals only had a mild scattering of lymphocytes in the myocardium and no myocardial damage (Fig. 1B).

A short course of FK506 also greatly reduced the intensity of the cellular infiltration in liver allografts. By day 7, the untreated animals had an intense mononuclear infiltration of the perportal areas and a diffuse infiltration of the sinusoids (Fig. 1C). The allografts in the treated animals had a mild-to-moderate periportal inflammation (Fig. 1D) that decreased spontaneously with time.

### DISCUSSION

The ACI and LEW strain combinations used for these experiments are strongly histoincompatible and differ for both MHC (RTI) and non-MHC histocompatibility genes. The effect of allograft acceptance under a longer course of FK506 therapy has been shown to be specific for the donor strain (5, 6). The same kind of short-course treatment, even as late as 4 days after transplantation, induced permanent acceptance of practically all liver grafts. The greater ease with which liver graft survival could be accomplished compared to the heart is consistent with much work showing a slight biologic advantage for hepatic grafts (21-24).

With either the liver or heart, the ability of FK506 to switch off the destructive immune process, practically on the eve of rejection, was extraordinary. The same thing has been accomplished occasionally in dog liver recipients with a few doses of antilymphocyte serum (25), and rather easily in canine recipients of livers and kidneys under FK506 treatment (11). However, liver graft acceptance in larger animals usually is not permanent. An explanation for permanent graft acceptance without further treatment may require revision of some of the classic theories of rejection and immunosuppression as has been discussed in another communication (18).

A conventional view might hold that FK506 disrupts the recognition and/or the early induction phase of the allograft

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**TABLE 1. Effect of FK506 on survival of heart grafts from ACI donors to LEW recipients**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>N</th>
<th>Graft survival (days)</th>
<th>MST (days)*</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>7</td>
<td>6, 6, 6, 6, 6, 7, 7</td>
<td>6.0</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>1.28 mg/kg days 0-13</td>
<td>6</td>
<td>56, 71, 80, 94, 96, 213&lt;sup&gt;c&lt;/sup&gt;</td>
<td>87.0</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>1.28 mg/kg days 0-13, then once a week for 5 weeks</td>
<td>12</td>
<td>8&lt;sup&gt;a&lt;/sup&gt;, 81, 82, 86, 87, 88, 88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.0</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>1.28 mg/kg days 0, 1, 2</td>
<td>6</td>
<td>23, 35, 36, 36, 58, 75</td>
<td>36.0</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>1.28 mg/kg days 4, 5, 6</td>
<td>6</td>
<td>23, 51, 89, 93, 98, 146</td>
<td>91.0</td>
<td>0.01</td>
</tr>
<tr>
<td>6</td>
<td>1.28 mg/kg days 5, 6, 7</td>
<td>6</td>
<td>25, 48, 49, 51, 60, 61</td>
<td>50.0</td>
<td>0.01</td>
</tr>
<tr>
<td>7</td>
<td>1.28 mg/kg days 3, 4, 5, 6</td>
<td>6</td>
<td>71, 71, 72, 76, 79, 81</td>
<td>74.0</td>
<td>0.01</td>
</tr>
<tr>
<td>8</td>
<td>2.5 mg/kg days 4, 5, 6</td>
<td>6</td>
<td>38, 54, 75, 85, 101, 120</td>
<td>80.0</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup> Median graft survival time in days.

<sup>b</sup> Group 2 vs. 3, 4, 5, 7, 8, N.S.; group 2 vs. 6, P=0.01.

<sup>c</sup> Died with pulsating graft.

**TABLE 2. Effect of FK506 treatment on survival of liver grafts from ACI donors to LEW recipients**

<table>
<thead>
<tr>
<th>Group</th>
<th>Days of treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>N</th>
<th>Graft survival (days)</th>
<th>MST (days)</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>10</td>
<td>9, 9, 9, 9, 10, 10, 10, 11, 12, 13</td>
<td>10.0</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Days 0, 1, 2</td>
<td>6</td>
<td>&gt;100×6</td>
<td>&gt;100</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>Days 2, 3, 4</td>
<td>6</td>
<td>9&lt;sup&gt;a&lt;/sup&gt;×100×5</td>
<td>&gt;100</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>Days 4, 5, 6</td>
<td>6</td>
<td>9, 10, 10, 12, &gt;100×10</td>
<td>&gt;100</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>Days 6, 7, 8</td>
<td>6</td>
<td>9, 9, 9, 10, 11, 12, 15</td>
<td>10.5</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>Days 3, 4, 5, 6</td>
<td>10</td>
<td>13&lt;sup&gt;a&lt;/sup&gt;×100×9</td>
<td>&gt;100</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup> FK506 was given by daily intramuscular injection (1.28 mg/kg/day).

<sup>b</sup> Death due to infection.
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FIGURE 1. A. Untreated heart graft at day 5 showing mononuclear infiltration and interstitial edema of the myocardium. B. FK506-treated heart graft at day 5; i.m. FK506 was started on day 4; note mild scattered mononuclear infiltration. C. Untreated liver graft at day 7 with intense mononuclear cell infiltration of portal tracts and sinusoids with liver cell necrosis. D. FK506-treated liver graft at day 7; i.m. FK506 was given on days 4, 5, and 6. Although there are mononuclear cells in the portal tracts with slightly scattered cells through the sinusoids, the recipient had permanent acceptance of the graft.

response and does not effect the proliferation of activated T cells, preventing thereby an amplification effect by cytokines and other mediators. Recent molecular biological study reveals that FK506 inhibits mRNA accumulation and transcription of early-phase T cell activation genes, such as IL-2, IL-3, IL-4, and gamma-IFN (26). If this explanation were true, it is hard to understand how treatment begun 4 or 5 days after exposure to the alloantigens could be so effective. The astonishing ability of FK506 to stop advanced and refractory rejection in human liver grafts (13) may be a clinical analogue to the animal observations.

REFERENCES


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