Therapy of Chronic Graft-\(\text{-}\)v-Host Disease in a Rat Model

By Georgia B. Vogelsang, Allan D. Hess, Kenneth J. Friedman, and George W. Santos

Chronic graft-\(\text{-}\)v-host disease (GVHD) is the most frequent late complication after allogeneic bone marrow (BMT) transplant. To test different treatments, we used a rat model of chronic GVHD which clinically, histologically, and immunologically resembles the disease occurring after human marrow transplant. The following treatments were administered: azathioprine 50 mg/kg/day plus prednisone 1 mg/kg/every other day; cyclosporine (CsA) 30 mg/kg/day; CsA 30 mg/kg/day plus prednisone 1 mg/kg/every other day; thalidomide 50 mg/kg/day; thalidomide 10 mg/kg/day; CsA 10 mg/kg/day; and thalidomide 10 mg/kg/day plus CsA 10 mg/kg/day. All drugs were administered by gavage. Half of the animals (six of 12) treated with azathioprine plus prednisone every other day responded to treatment. The majority of animals (seven of 12) treated with CsA died early. The addition of prednisone every other day did not improve survival. The surviving animals treated with CsA (with or without prednisone) responded to treatment. Half of the animals treated with each of the above regimens had recurrent or progressive disease after therapy was discontinued. Thalidomide treatment was successful in the majority of animals (16 of 18). The addition of low-dose CsA to low-dose thalidomide resulted in a faster rate of response (12.1 ± 1.8 vs 28.2 ± 1.6 days). We conclude that both thalidomide and thalidomide plus CsA appear promising in this model as treatment for chronic GVHD.

C H R O N I C graft-\(\text{-}\)v-host disease (GVHD) is the most frequent late complication after allogeneic bone marrow transplant (BMT). Chronic GVHD is a multisystem disorder which resembles many naturally occurring autoimmune disorders. Despite improved prevention and treatment of acute GVHD, chronic GVHD still occurs in 30% to 60% of patients receiving allogeneic BMT who survive >100 days. Patients have frequently returned home by the time their chronic GVHD becomes evident, making detection of chronic GVHD and assessment of therapy difficult.

We used an established rat model to evaluate therapy of chronic GVHD. In this model, rats develop a clinical picture similar to that of humans, with thickened skin, alopecia, dry mouth, dry eyes, hepatic disease, and immunodeficiency due to nonspecific suppressor cells. The present report details our results with this model comparing azathioprine plus prednisone, cyclosporine (CsA) with or without prednisone, and thalidomide with or without CsA treatment.

MATERIALS AND METHODS

Female Lewis (RTI-l) and ACI (RTI-a) rats aged 12 weeks were purchased from Sprague Dawley, Indianapolis.

Drugs and irradiation. Thalidomide (administered at a dose of 10 or 50 mg/kg/day, gift of Dr S. Sampao, Sao Paulo, Brazil), azathioprine (administered at a dose of 1.5 mg/kg/day, Burroughs Wellcome, Research Triangle Park, NC), prednisone (administered at a dose of 1 mg/kg/every other day, Lederle Laboratories, Wayne, NJ), and cyclosporine [administered at a dose of 10 mg/kg, 2.5 mg/kg subcutaneous (SC) equivalent] or 30 mg/kg, 7.5 mg/kg SC equivalent, every day (gift of Sandoz, Basel, Switzerland) were prepared as a suspension in vegetable oil and administered by gavage. Total body irradiation (TBI) was delivered with a dual-source \(^{137}\)Cs animal irradiator at 110 rad/min.

BMT. Recipient animals received TBI at 1,050 rad, and 60 × 10\(^6\) donor cells were infused in 1 mL by tail vein 24 hours later. All animals received medicated antibiotic water (Septra, gift of Burroughs Wellcome, Research Triangle Park, NC), prednisone (administered at a dose of 1 mg/kg/every other day, Lederle Laboratories, Wayne, NJ), and cyclosporine [administered at a dose of 10 mg/kg, 2.5 mg/kg subcutaneous (SC) equivalent] or 30 mg/kg, 7.5 mg/kg SC equivalent, every day (gift of Sandoz, Basel, Switzerland) were prepared as a suspension in vegetable oil and administered by gavage. Total body irradiation (TBI) was delivered with a dual-source \(^{137}\)Cs animal irradiator at 110 rad/min.

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Table 1. Results of Therapy for Chronic GVHD

<table>
<thead>
<tr>
<th>Evaluable Animals</th>
<th>Therapy</th>
<th>Complete Response</th>
<th>Partial Response</th>
<th>No Response (Deaths)</th>
<th>Mean Time to Response ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>12 (3)</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>Azathioprine + prednisone</td>
<td>3</td>
<td>3</td>
<td>6 (3)</td>
<td>34 ± 1.4</td>
</tr>
<tr>
<td>12</td>
<td>CsA</td>
<td>4</td>
<td>1</td>
<td>7 (7)</td>
<td>31 ± 2.4</td>
</tr>
<tr>
<td>12</td>
<td>CsA + prednisone</td>
<td>5</td>
<td>0</td>
<td>7 (7)</td>
<td>30.8 ± 3</td>
</tr>
<tr>
<td>18</td>
<td>Thalidomide</td>
<td>15</td>
<td>1</td>
<td>2 (2)</td>
<td>28.2 ± 1.6</td>
</tr>
<tr>
<td>3</td>
<td>Low-dose CsA</td>
<td>0</td>
<td>0</td>
<td>3 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>Low-dose thalidomide</td>
<td>0</td>
<td>0</td>
<td>3 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>Low-dose CsA + thalidomide</td>
<td>9</td>
<td>1</td>
<td>2 (2)</td>
<td>12.1 ± 1.8</td>
</tr>
</tbody>
</table>

Animals were treated once chronic GVHD was diagnosed clinically and histologically. Deaths reported occurred during the 40 days of therapy and are included in the no response animals as shown by ( ). Animals with complete responses had total resolution of clinical and histologic findings of GVHD. Animals with partial responses had at least a 50% improvement of their GVHD. The time to response represents the first day of treatment that a clinical improvement (hair growth) in chronic GVHD was noted in responding animals.

Abbreviations: SD, standard deviation; NA, not applicable.

roughs Wellcome), neomycin G (Sigma Chemical, St Louis), and erythromycin (Barr Laboratories, Northvale, NJ). Once acute GVHD was diagnosed, low-dose thalidomide (10 mg/kg/day for 40 days) was administered. At completion of therapy, all animals had resolved their acute GVHD. Several weeks after completion of therapy, 10% of animals developed chronic GVHD as documented by clinical and histologic appearance as described previously. Biopsies were obtained at the start of therapy, at completion of therapy, and every 2 weeks subsequently until the animals appeared clinically stable for 4 weeks. Animals were also biopsied for any signs of recurrent disease.

**Grading of response.** Animals with a complete response had a return to pretransplant weight with resolution of alopecia, bilirubinuria (when present), and sclerotic collagen on serial skin biopsy. Animals with a partial response failed to resolve all of the above criteria, but had at least a 50% improvement as judged by clinical status and histology of skin biopsy. Animals with no change or progressive disease were graded as no response. To document chronic GVHD further, we performed autopsies on the no-treatment animals and animals dying during therapy.

**Statistics.** The main statistical endpoints of this study were time to recovery and time to death. Event time distributions were estimated by the method of Kaplan-Meier and compared by the log-rank statistic. For time-to-recovery analyses, death or end of study was a censoring event. For time-to-death analyses, recovery or end of study was a censoring event.

**RESULTS**

**No treatment.** Twelve animals received no therapy for chronic GVHD. Three animals died of progressive chronic GVHD. The remainder survived the entire 6-week therapy trial. These animals were killed, and autopsy findings were consistent with chronic GVHD. Results for all treatment groups are summarized in Table 1.

**Azathioprine and prednisone.** Administration of azathioprine 1 mg/kg/day plus prednisone 1 mg/kg/every other day resulted in complete responses in three animals, partial responses in three animals, no response in three animals, and the death of three animals. For the animals with no or partial responses, therapy was continued for 40 days more. These animals had no further improvement. When therapy was stopped (at 40 days for complete responders and at 80 days for partial responders), half of the animals had recurrence or progression of chronic GVHD.

**CsA.** Animals that received CsA had an acute worsening of their disease with increased erythema. Most of the animals (seven of 12) died during the first 2 weeks of therapy. These animals had a significantly worse survival as compared with other groups \((P = .05)\). Autopsy of these animals showed chronic GVHD (seven/seven) and pneumonitis (five/seven). All surviving animals responded to treatment. Addition of 1 mg/kg prednisone every other day did not decrease the early toxicity of this therapy. Once cyclosporine was discontinued, chronic GVHD recurred in six of ten animals.

**Thalidomide.** Of 18 thalidomide-treated animals, 15 had complete resolution of chronic GVHD. Two animals died during the first week of therapy. Hair regrowth first appeared around the head and proceeded rapidly down the trunk. Serial biopsies showed progressive collagen loss and progressive increase in dermal appendages (sweat glands and hair follicles). The animals with the most severe chronic GVHD at start of therapy as manifested by skin ulceration had residual alopecia clinically and persistent sclerotic collagen histologically. These findings resolved after completion of thalidomide. One animal that showed no resolution of alopecia had loss of sclerotic collagen on serial biopsy but had no return of hair follicles. After therapy was discontinued, thalidomide-treated animals showed no return of disease when followed for a minimum of 6 months. Representative photographs of animals before and after therapy and the corresponding biopsies are shown in Figs 1 and 2.

Three animals received low-dose CsA (10 mg/kg/day),
and three animals received low-dose thalidomide (10 mg/kg/day). During the 40-day treatment, no response was noted. Low-dose thalidomide (10 mg/kg) and CsA (10 mg/kg) were administered together. The early deaths were also similar to those which occurred with thalidomide alone. All animals remained stable after therapy was discontinued. The striking feature in these animals was the rapid tempo of response. As shown in Fig 3, animals receiving the combination of low-dose thalidomide and CsA had a more rapid response to treatment (as measured by first-day hair regrowth) than any other treatment group \((P < .001)\). No other significant differences among groups was noted.

DISCUSSION

Chronic GVHD remains a significant problem in allogeneic BMT. Chronic GVHD is the most common late complication of BMT, and continues to occur at about the same incidence despite improvements in prevention of treat-
Thus, the number of animals in each treatment group was low. Moreover, these animals were sick, and despite the use of prophylactic antibiotics, one fourth of the animals died during the first 2 weeks of therapy and had a statistically worse survival than the other treatment groups.

In this study, a rat model was used to explore different therapies for chronic GVHD. This model of chronic GVHD has previously been well characterized histologically and immunologically. This model uses a major histocompatibility-mismatched BMT. Although as many as 20% of the animals developed chronic GVHD in some transplants, in most transplants 10% of the animals developed chronic GVHD. Thus, the number of animals in each treatment group was low. Moreover, these animals were sick, and despite the use of prophylactic antibiotics, one fourth of untreated control animals died during a 6-week period.

Use of azathioprine and prednisone in this model produced results similar to those reported for patients receiving the same therapy. The results with CsA, however, were somewhat worse than those in human trials. Most of the animals died during the first 2 weeks of therapy and had a statistically worse survival than the other treatment groups. Autopsies of these animals showed severe chronic GVHD and usually pneumonitis. Thus, infection appears to be the main reason that these animals did not do as well as patients treated with a similar regimen. Steroids did not alter the early toxicity seen with CsA. The high number of animals with recurrent chronic GVHD was disappointing. A longer duration of therapy and/or tapering of the CsA may result in fewer recurrences of chronic GVHD. Three animals were rescued with thalidomide after relapsing off CsA (data not shown).

Thalidomide is an interesting "new" immunosuppressant useful in prophylaxis and treatment of acute GVHD in the rat model. We have now treated >100 animals with acute GVHD with thalidomide. Only two animals that died early had residual acute GVHD at autopsy. Adoptive transfer experiments using thalidomide-treated chimeras suggest that specific suppressor cells had been induced, since animals receiving original donor strain marrow plus chimeric lymphocytes did not develop GVHD whereas animals receiving third-party marrow plus chimeric lymphocytes did. To determine if thalidomide had any effect in chronic GVHD, we administered it to the animals. Because of the difficulty in producing these animals, the optimal dose of thalidomide from acute GVHD studies was chosen for use in these studies. No formal dose response studies were performed. Thalidomide was well tolerated without the high incidence of early deaths which occurred in CsA-treated animals. Most animals receiving thalidomide had complete resolution of their chronic GVHD. A few animals receiving thalidomide with or without other agents had persistent alopecia. These animals appear to have lost their hair follicles and may have been incapable of regrowing hair.

Thalidomide was combined with prednisone and with azathioprine to determine if the combinations of therapy had any effect on the tempo of response or toxicity. There was no discernible change in animals receiving combination therapy as compared with animals receiving thalidomide alone (data not shown).

Previous work with prophylaxis of acute GVHD has suggested that thalidomide and CsA may be additive or synergistic when given together in low doses. The final set of animals received low doses of both drugs. As compared with animals receiving thalidomide, the combined therapy with prednisone or azathioprine, or CsA with or without prednisone, responses in animals receiving thalidomide plus cyclosporine were more rapid, with definite improvement in clinical appearance noted ten to 14 days after initiation of therapy. In previous experiments, the plasma levels of each drug in animals receiving low doses of either drug or both drugs did not change, making it unlikely that alterations in drug metabolism resulting in increased drug levels accounted for this effect. The mechanisms of this synergism between CsA and thalidomide is unknown. A fluorescent CsA derivative and thalidomide derivative identify the same lymphocyte populations, both by phenotypic and functional analysis. Preliminary experiments have also suggested that at least two of the intracellular proteins which bind thalidomide also
bind CsA. Why these drugs, which one would expect to be competitive based on these data, appear to have additive or synergistic effects when administered together is not clear. Currently, laboratory studies involve phenotypic and functional analysis of lymphocytes from animals receiving CsA, thalidomide, and the combination of the two to try to determine at a cellular level the mechanism of synergy. Further work on the intracellular binding proteins is also underway.

These animal experiments suggest that thalidomide is an active agent in treatment of chronic GVHD. The drug is tolerated well, and most animals totally resolved their disease, with no relapses observed once therapy was completed. An exciting finding is that addition of CsA to thalidomide improved the rate of response with acceptable mortality and morbidity. These studies also suggest that autoimmune disorders similar to the disease processes in chronic GVHD may be reversible with similar therapy. Future work will be directed at understanding the mechanism of action of both drugs singly and together in treatment of chronic GVHD.

REFERENCES


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