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Prolonged Prevention of Acute Graft-Versus-Host Disease After Allogeneic Bone Marrow Transplantation by Donor Pretreatment Using FK 506

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ACUTE graft-versus-host disease (GVHD) is a systemic manifestation of activation of normal allodisparate transplanted lymphoid cells to recipient epithelial target organs. In clinical bone marrow transplantation (BMT) between HLA identical siblings, acute GVHD occurs in up to 50% of cases,[1] many times during the early posttransplant period. It has recently been shown that FK 506 is superior to cyclosporine (CyA) for the prevention of lethal acute GVHD after allogeneic bone marrow transplantation in the rat.[2] It is known that FK 506 compared to CyA in vitro has a higher potency with regard to its effects on the early events of T-cell activation, particularly if FK 506 is present at or before the time of antigenic stimulation. We have examined whether a single treatment of the allogeneic donor, either alone, in combination with continuous recipient prophylactic therapy, or with discontinuous recipient therapy, could significantly prolong the GVHD-free interval and subsequent overall survival after fully allogeneic bone marrow transplantation in our previously described animal model.[2]

MATERIALS AND METHODS

Animals

Male Lewis (*RT-1^l*) and ACI (*RT-1^a*) rats (Harlan Sprague Dawley, Indianapolis, Ind), aged 7 to 9 weeks, were used in all experiments. Animals were maintained in a laminar flow caging system (Thorens Caging Systems) and given acidified water as described previously.[2]

BMT and Assessment of GVHD

BMT was performed according to a protocol previously described at our institution.[2] Briefly, bone marrow and spleen cells were prepared as single-cell suspensions. Sixty million ACI bone marrow and 30 million spleen cells were injected via the penile vein into lethally irradiated (1000 rad) Lewis recipients. All animals received antibiotics in the immediate posttransplant period. All animals were observed daily for evidence of GVHD. Clinical and histological criteria (as assessed by weekly ear biopsies) used for the diagnosis of GVHD, have been described in a previous study from our institution.[2]

Immunosuppression

FK 506 was a gift from Fujisawa Pharmaceutical Co (Osaka, Japan). The powder with carrier solvent was diluted in normal saline for IM injections. FK 506 was given once a day, unless otherwise specified.

Study Design and Statistical Analysis

The allocation of groups is shown in Table 1. Groups of animals received an allogeneic bone marrow graft from donor animals that had either received either one dose of FK 506 or no drug. The respective recipients received either no additional therapy, a course of FK 506 starting on day 0, or a course of FK 506 starting on day 3. The GVHD-free interval and survival were compared between groups. Statistical analysis was performed using Student's t test. $P < .05$ was considered significant.

Table 1. Group Assignments Showing Donor and Recipient Treatments

Group	n	Donor Treatment (Days)	Recipient Treatment (Days)
A	10	0	0–6
B	8	0	3–8
C	8	1	0
D	10	1	0–6
E	8	1	3–8
F	8	0	0

All FK 506 doses were 1 mg/kg per day IM.

RESULTS

The mean GVHD-free interval and survival are shown in Table 2. The addition of one dose of FK 506 (1 mg/kg) significantly prolonged the mean GVHD-free survival and survival when compared to no donor pretreatment (P = .01). When donor pretreatment was added to a 0- to 6-day course of FK 506 (1 mg/kg) given to the recipient, and compared to a recipient 0- to 6-day course of FK 506 alone, the GVHD-free interval was extended from 42 to 60 days ($P < .001$). When donor pretreatment was combined with a 3- to 8-day course of FK 506 given to the recipient, the GVHD-free interval was again prolonged in comparison to a recipient course alone, although by a smaller degree than when the recipients had received a 0- to 6-day course of therapy. When the recipients received no therapy, the addition of one dose of FK 506 given to the donor was, by itself, sufficient to prolong the GVHD-free interval. The differences in mean GVHD-free intervals between these

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Table 2. Mean Time to GVHD and Mean Survival

Group	Mean Time to GVHD (Days Post-BMT)	Mean Survival (Days Post-BMT)
A	42.0 ± 3.1	45.0 ± 4.6
B	45.0 ± 4.6	48.0 ± 5.1
C	12.7 ± 1.3	18.8 ± 0.87
D	60.0 ± 5.9	64.0 ± 6.13
E	52.0 ± 4.8	59.0 ± 6.2
F	10.0 ± 1.1	15.0 ± 1.7

All values are means ± SEM.

groups were reflected in the differences of mean survival times (Table 2).

DISCUSSION

In this study, we have shown that the addition of one dose of the immunosuppressant, FK 506, to an allogeneic bone marrow donor can significantly prolong the mean GVHD-free interval after allogeneic BMT. This donor pretreatment therapy gives better results when combined with a recipient course of FK 506 from days 0 to 6 rather than 3 to 8, and it may be that continuous therapy is more beneficial than fragmented prophylaxis. The most likely explanations for these observations are drug carry-over effects or residual inhibitory affects on T cells. Drug carry-over effects are the most likely explanation, since it became known that FK 506 is difficult to remove from in vitro systems.[3] The alternative possibility is a direct effect on the donor cells prior to BMT. Perhaps it is possible that this question could be addressed by measuring donor blood levels of FK 506 and waiting until these return to zero. This would then eliminate carry-over effects and allow assessment of whether pretreatment of the cells gives additional immunosuppressive activity. There is also the prospect of recipient pretreatment for solid organ allografting, and whether this is valuable in reducing incidence or severity of allograft rejection. FK 506 has been shown to be effective in delaying the onset of rejection in the heterotopic heart allograft model in the rat.[4] Therefore, we conclude that pretreatment of an allogeneic donor prior to BMT with FK 506 can prolong the GVHD-free interval and survival, particularly when this treatment is combined with continuous recipient therapy.

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