1258

The Induction of Pseudo–Graft-Versus-Host Disease Following Syngeneic Bone Marrow Transplantation Using FK 506

M.H. Cooper, G.G. Hartman, T.E. Starzl, and J.J. Fung

CYCLOSPORINE (CyA)-induced pseudo–graft-versus-host disease (GVHD) developing in syngeneic rat bone marrow chimeras has features resembling classical acute and later chronic GVHD in the fully allogeneic bone marrow chimera.[1] These features develop in the early post-CyA period, and usually result in a mild, acute form of GVHD, followed by a more chronic autoimmune-like syndrome in a high percentage of animals, particularly with high doses of CyA.[1] CyA-induced thymic changes, which include disruption of thymocyte maturation while on therapy, are thought to be the mechanisms responsible for these effects following the discontinuation of CyA therapy.[1] Since FK 506, like CyA, affects the thymic microenvironment,[2] we decided to observe the nature of any pseudo-GVHD reactions induced by the abrupt assation of FK 506 therapy following syngeneic BMT in the Lewis rat.

MATERIALS AND METHODS

Animals

Male Lewis rats (Harlan Sprague Dawley, Indianapolis, Ind), ages 6 to 8 weeks, were used in all experiments. Animals were maintained in a laminar flow caging system (Thorens Caging Systems), given acidified water containing tetracycline hydrochloride (100 mg/L) and neomycin sulfate (10 mg/L) and fed rat chow ad libitum.

Immunosuppressants

FK 506 was a gift from Fujisawa Pharmaceutical Co (Osaka, Japan). The powder with carrier solvent, HCO-60 and D-mannitol was diluted in normal saline for IM injection. FK 506 was given once per day unless otherwise specified.

BMT and GVHD

Animals were transplanted in accordance with our previously described protocol.[3] Briefly, bone marrow was harvested from femurs and tibias of donor Lewis animals, washed and resuspended in Hanks' balanced salt solution, supplemented with gentamycin (50 mg/mL). A total of 60×10^6 BM cells were infused to lethally irradiated (1000 rad) Lewis recipients via the penile vein 2 hours following irradiation. All animals received antibiotics in the immediate postoperative period. Lethally irradiated animals who received no marrow replacement, served to test adequacy of the preparative regime. Clinical GVHD was assessed in all animals on a daily basis. Both clinical and histological GVHD were defined according to previously described criteria from our institution.[3]

Study Design

Groups of 8 rats were treated with various doses of FK 506 for between 21 and 28 days following BMT, starting on the day of BMT. The duration of therapy, dose of FK 506, and group assignments are as shown in Table 1. The incidence of GVHD, mean GVHD-free interval, and overall survival were assessed for each group.

Table 1. Group Assignments

Group	Reconstitution	FK 506 (mg/kg per day)	Treatment Period (d)
A	Lewis	1.0	21
B	Lewis	0.5	28
C	Lewis	0.25	28

RESULTS

The type, onset of GVHD, and survival consequent upon the abrupt cessation of FK 506 at different times after syngeneic bone marrow transplantation in our rat bone chimeras are shown in Table 2. All irradiation controls died within 12 days of irradiation.

In all groups, at least 75% of animals developed acute GVHD, as evidenced by dermatitis, red ears, and unkempt appearance. Acute GVHD increased with increasing doses of FK 506 (Table 2). The acute GVHD-free interval was, however, prolonged at higher FK 506 doses, and ranged from 40 to 55 days following BMT. The number of animals that developed a more severe, acute GVHD reaction, which led to the early features of chronic GVHD, were between 60% and 75%, after mean times ranging from 65 to 78 days after BMT.

Overall survival in the syngeneic chimeras did not fall

Table 2.

Group	Percent With Acute GVHD	Mean Time to Acute GVHD (Days After BMT)	Mean Time to Chronic GVHD (Days After BMT)	Percent Survival Posttransplant
A	90	40 ± 3.0	65 ± 5.3	90
B	80	55 ± 2.8	71 ± 6.9	100
C	75	49 ± 2.9	78 ± 6.9	100

All values are means ± SEM.

From the Departments of Surgery (M.H.C., T.E.S., J.J.F.) and Pathology (G.G.H.), University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania.

Supported by Research Grant No. DK 29961, National Institutes of Health, Bethesda, Maryland.

Address reprint requests to Dr M.H. Cooper, Department of Surgery, Division of Transplantation, University of Pittsburgh, 15th Floor, Biomedical Science Tower, Pittsburgh, PA 15232.

© 1991 by Appleton & Lange

0041-1345/91/$3.00/+0

below 90% at the end of the follow-up period (Table 2), which was 90 days from BMT. The 10% mortality in group A was related to poor general condition of these animals at the time of development of the chronic syndrome.

DISCUSSION

We have shown that FK 506 can induce a form of pseudo-GVHD. The histological features confirm our clinical impressions that the nature of the induced GVHD is severe in the syngeneic recipients and affects survival at high doses. Interestingly, in allogeneic recipients, this syndrome is fatal in a high percentage of animals when a high dose of FK 506 is given for a relatively long period and then stopped (data not shown). The mechanisms shown to be responsible for the development of CyA-induced pseudo-GVHD are related to how CyA affects the thymic microenvironment and thymocyte maturation; ie, it inhibits the production of single positive mature thymocytes from the double-positive thymocyte stage.[4] FK 506 also causes this defect. This pseudo-GVHD compares well with that described with CyA. It is, however, more severe in terms of severity of subsequent pseudo-GVHD, and this affects survival in syngeneic recipients, given high doses of FK 506.

REFERENCES

1. Hess AD, Fischer AC, Beschorner WE: J Immunol 145:526, 1990

2. Pugh-Humphreys RGP, Ross CSK, Thomson AW: Immunology 70:398, 1990

3. Markus PM, Cai X, Ming W, et al: Transplantation (in press)

4. Kosugi A, Sharrow SO, Shearer GM: J Immunol 142:3026, 1989