LING ZHI-8 (LZ-8) is a protein from the mycelial extracts of *Ganoderma lucidum* and has immunomodulatory capacities. Formerly it was reported to be mitogenic toward mouse splenocytes and suppressive in vivo by reducing HBsAg-specific antibody production and by preventing the incidence of diabetes in NOD mice. To specify possible clinical use of LZ-8, the mitogenic effects of LZ-8 were tested in the presence of human mononuclear cells (MNC) and T lymphocytes, as well as suppressive capacities of LZ-8 in vitro in an MLC with MNC or T lymphocytes and Epstein-Barr Virus-transformed (EBV) B cells. The immunosuppressive effects of LZ-8 were also investigated in a model of allogeneic mouse skin transplantation and in a model of allografted rat pancreatic islets.

**MATERIALS AND METHODS**

**Mitogenic Activity of LZ-8**

Human MNC or purified T cells were incubated with 3 LZ-8 concentrations (0.1, 1, and 10 μg/mL) for 3, 4, 5, 6, and 7 days.

**Immunosuppressive Activity of LZ-8 In Vitro**

Human T cells were incubated with irradiated allogeneic EBV-B cells and LZ-8 in three concentrations (0.1, 1, and 10 μg/mL) for 6 days.

**Mouse Skin Transplantation**

B10.D2 mice (H-2d) served as skin donors and C57Bl10 mice (H-2b) as recipients. Full-thickness skin flaps were attached to the flank of recipients after removal of a corresponding skin area. Rejection occurred on the day of complete necrosis of the transplanted skin. Group 1 (controls, n = 12) received an injection of saline, twice per week; group 2 (n = 11) received 15 mg/kg LZ-8, twice per week; and group 3 (n = 12) received 7.5 mg/kg LZ-8 four times per week.

**Rat Pancreatic Islet Transplantation**

Lewis rats (RT-1<sup>H</sup>) were the donors of pancreatic islets and diabetic (streptozotocin IV) F344 rats (RT-1<sup>H</sup>) were the recipients of two donor islet grafts. Pancreatic islets were obtained after intraductal distension with collagenase, stationary digestion, filtration, and density gradient centrifugation, as published earlier. Rejection occurred on the day of complete necrosis of the transplanted skin. Group 1 (controls, n = 9) received no postoperative immunosuppression. Group B (n = 6) received an injection of 15 mg/kg LZ-8 twice per week posttransplant and group C (n = 6) 5 mg/kg LZ-8 daily. Group D (n = 6) had continuous LZ-8 injection by an osmotic minipump in a concentration of 5 mg/mL with a volume of 2 mL and an operational period of 7 days.

**RESULTS**

**Mitogenic Activity of LZ-8**

A strong mitogenic response was observed in all incubations of LZ-8 with human MNC. Peak activity was measured after 3 days of incubation with 1 μg/mL LZ-8. The stimulatory activity decreased rapidly for all LZ-8 concentrations after 4 days. In the absence of monocytes, there was hardly any LZ-8-induced stimulation of human T cells.

**Immunosuppressive Activity of LZ-8 In Vitro**

The mitogenic response of LZ-8 on human MNC, containing monocytes, overruled the possible immunosuppressive effects of LZ-8 in a MLC. In a modified MLC with T cells and allogeneic EBV-B cells, significant suppression of T-cell activation was achieved by LZ-8. Addition of 0.1 μg/mL LZ-8 resulted in 42% inhibition, 1 μg/mL in 53%, and 10 μg/mL in 66% inhibition.

**Mouse Skin Transplantation**

Administration of LZ-8 led to increased mean survival times (MST) of allogeneic mouse skin. MST ± SD were, respectively: 10.2 ± 1.1 days in group 1 (controls), 11.5 ± 1.8 days in group 2; and 13.3 ± 2.9 days in group 3 (group 3 vs 1: P < .01).

**Rat Pancreatic Islet Transplantation**

Treatment with LZ-8 resulted in markedly prolonged graft survival. Group A (controls) rejected their islet grafts after 4.7 ± 0.15 days. MST ± SD of transplanted islets in group B was 9.7 ± 0.8 days and in groups C and D 11.0 ± 0.7 days and 12.5 ± 1.2 days, respectively (groups B, C, D vs A: P < .01; and B vs D: P < .05).

**DISCUSSION**

LZ-8 proves to have paradoxical immunomodulating effects. In the presence of monocytes, a strong mitogenic response on human MNC by LZ-8 was obtained. Evident...
immunosuppression by LZ-8 was demonstrated on the proliferative response of T cells with EBV-B cells in the absence of monocytes. Also, in both tested in vivo allogeneic transplantation models, significant improvement of MST was achieved by LZ-8 in comparison with controls. No toxic side effects of LZ-8 could be discerned in these studies. Future studies should address exact modes of action of LZ-8.

REFERENCES