Ling Zhi-8: A Fungal Protein With Immunomodulatory Effects

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LING ZHI-8 (LZ-8) is a protein from the mycelial extracts of *Ganoderma lucidum* and has immunomodulatory capacities.[1] Formerly it was reported to be mitogenic toward mouse splenocytes and suppressive in vivo by reducing HBsAg-specific antibody production[2] and by preventing the incidence of diabetes in NOD mice.[3] 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 ($H\text{-}2^d$) served as skin donors and C57Bl10 mice ($H\text{-}2^b$) 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 ($RT\text{-}1^l$) were the donors of pancreatic islets and diabetic (streptozotocin IV) F344 rats ($RT\text{-}1^{lv}$) 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.[4] Rejection occurred on the first of 3 days with blood glucose >11 mmol/L. Group A (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

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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 improval 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.

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