

Osteogenesis in xenogeneic bone transplantation, using an immunosuppressant

Rabbit-rat experiments

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We investigated osteogenesis and lymphocyte subsets in xenogeneic bone transplantation, using the immunosuppressant FK506 (FK). Iliac bones of rabbits were transplanted as fresh and frozen xenogeneic bone grafts into an intramuscular pouch of rats. FK was injected intramuscularly in half of the rats in a dose of 3 mg/kg/day for 14 days after transplantation. At 2, 4, and 8 weeks, transplanted grafts and the lymphocyte subsets of these rats were examined. In the group not given FK, the grafted

bone became necrotic and infiltrated with small round cells around the trabeculae. In the FK group, at 2 and 4 weeks, new bone was formed in the fresh xenografts without infiltration of lymphocytes. At 8 weeks, the new bone became necrotic and lymphocytes were present. The percentage of T cells (CD 5), B cells and the ratio of CD 4 cells/CD 8 cells were smaller in the FK group. Using an immunosuppressant we concluded that xenogeneic bone has an osteogenic potency.

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It has been confirmed repeatedly that xenogeneic bone has no osteogenic potency and allografts also are inferior to autografts (Chalmers 1959). Like allogeneic bone, xenogeneic bone provokes an immunologic reaction causing rejection of the graft (Bassett et al. 1962, Hallen 1966). In an attempt to suppress the immunologic reaction to fresh bone allografts, immunosuppressive agents have been used (Burchardt et al. 1981, Goldberg et al. 1984). Little is known about the antigenicity of xenogeneic bone and its immunosuppression. In 1984, FK 506 (Fujisawa Pharmaceutical Co., Japan) was found to be a strong immunosuppressive agent. Treatment with FK prolonged xenograft rejection of the skin, heart (Ochiai et al. 1987) and liver (Valdivia et al. 1991), but there have been no reports of its use in xenogeneic bone transplantation.

We investigated whether xenogeneic bone transplanted intramuscularly can form new bone in the presence of FK. We also analyzed the immune responses of xenogeneic bone grafts, using lymphocyte subsets.

Material and methods

Osteogenesis of xenogeneic bone

The xenogeneic donors were male Japanese white

rabbits weighing about 2 kg. The recipients were male Wistar rats weighing about 300 g. We studied 30 fresh grafts and 30 frozen grafts, both groups being divided into 15 rats given FK and 15 rats not given FK:

- a) Fresh xenograft with FK.
- b) Fresh xenograft without FK.
- c) Frozen xenograft with FK.
- d) Frozen xenograft without FK.

Fresh xenograft groups (a, b). A cube of cancellous bone measuring about 5 mm was collected from the ilium of the rabbits under anesthesia and used as the xenograft. The xenogeneic bone was transplanted into an intramuscular pouch in the back of the rats.

In the FK group, FK was injected intramuscularly in a dose of 3 mg/kg/day for 14 days after transplantation.

Frozen xenograft groups (c, d). Frozen xenogeneic bone stored at -40 °C for 7 days was transplanted into the muscle pouch of rats in the same way, and the rats were divided into groups with or without FK. In the FK group, FK was administered as above.

At 2, 4, and 8 weeks after transplantation, 5 rats were collected from each group. The transplanted bone was excised together with the surrounding tissue. This sample was fixed in 10% buffered neutral formalin, demineralized with formic acid, stained with hematoxylin and eosin and then examined histologically.

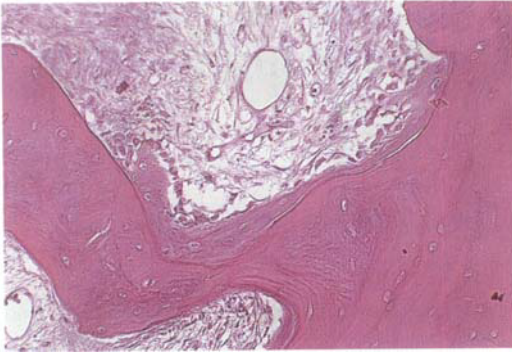


Figure 1. New bone formation was observed at 2 weeks in the FK group after transplantation of a fresh xenograft. No infiltration of small round cells was seen (HE $\times 100$).

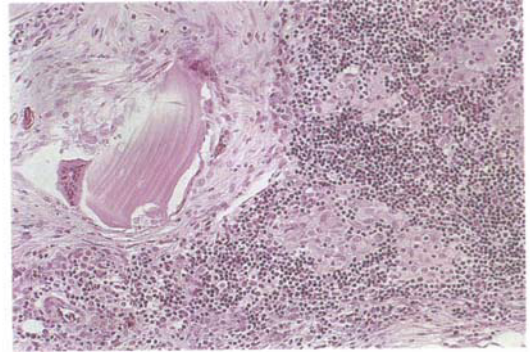


Figure 2. Fresh xenograft in the group without FK at 2 weeks. Small round cell infiltration and no new bone formation were observed (HE $\times 100$).

Analysis of lymphocyte subsets

At 2, 4, and 8 weeks after transplantation, peripheral blood samples were collected from the fresh xenograft groups, and the control group at day 0 (5 FK rats and 5 no FK rats). Lymphocyte subsets were determined by flow cytometry using monoclonal antibodies. The monoclonal antibodies used were FITC-labeled W3/25 (CD4+ = helper T cell), S/C (CD8+ = cytotoxic T cell), and non-labeled leucosialin (CD5+ = pan T), LCA (B cell); all these were products of Serotec Co.

In addition, the ratio of CD4+ cells to CD8+ cells was determined for each positive cell. The Kruskal-Wallis test was used to compare the 2 groups with the control group. When significant differences were found, the Wilcoxon test was used.

Results

Osteogenesis of xenogeneic bone

In the FK group with a fresh graft, new bone formation was observed around the trabeculae of the grafts in all specimens at 2 weeks after transplantation. No cellular infiltration was observed and the bone marrow had become fibrotic (Figure 1). At 4 weeks after transplantation, all specimens exhibited new bone formation around the trabeculae, and the volume of newly formed bone was more marked than at 2 weeks.

At 8 weeks, new bone in the graft became necrotic and small round cell infiltration around the bone was observed in 4 of the 5 specimens. There was no cell infiltration in the remaining specimen.

In the group without FK but with a fresh graft, no osteogenesis was observed at 2, 4, or 8 weeks after transplantation. After 2 weeks, every specimen

showed necrosis of the trabeculae and marked small round cell infiltration (Figure 2).

In the frozen groups with and without FK, the trabeculae were necrotic and new bone formation was not observed at 2, 4, or 8 weeks after transplantation. In the group with FK, small round cell infiltration was not observed until after 2 weeks. In the group without FK, marked cell infiltration was observed.

Lymphocyte subsets (Table 1)

The fraction of CD5+ cells in the group with FK changed from an initial median of 70% to 57% 2 weeks after transplantation. There was a difference ($p < 0.05$) in the population of CD5+ cells at 2 and 4 weeks after transplantation between the groups with and without FK.

At 4 weeks, there was an increase ($p < 0.05$) in the ratio of CD4+ cells to CD8+ cells in the group with FK vs. the group without FK.

Table 1. The population of CD5+ cells and B cells in peripheral blood (percent) and the ratio of CD4+ cells to CD8+ cells. Values are median and range

	Weeks	no FK	p	FK
CD5	0 (control)	69 (58-74)		70 (64-75)
	2	72 (64-82)	0.01	57 (55-61)
	4	72 (63-79)	0.03	58 (56-60)
	8	73 (69-82)	0.1	64 (59-68)
CD4/CD8	0 (control)	2 (2-2)		2 (2-2)
	2	2 (2-3)	0.5	2 (2-2)
	4	2 (2-3)	0.04	2 (1-2)
	8	2 (2-2)	0.5	2 (2-2)
B	0 (control)	21 (17-27)		24 (18-26)
	2	28 (18-29)	0.04	12 (9-22)
	4	26 (21-36)	0.01	10 (6-14)
	8	28 (20-37)	0.02	12 (4-21)

The increase in the population of B cells at 2 weeks differed significantly from that in the control. Differences in the population of B cells were found between the groups with and without FK at 2, 4, and 8 weeks ($p < 0.05$).

Discussion

The increased demand for organ transplantation calls for a reexamination of xenografts.

Xenogeneic perichondrium has been transplanted to cartilage defects in rabbit knees, and the graft survived with formation of hyaline cartilage (Homminga et al. 1991). But xenogeneic bone is antigenic and sets up an immune reaction in the host tissue, and the bone is either sloughed or absorbed (Hallen 1966).

The mouse without a thymus lacks mature T lymphocytes and the ability to reject some allografts and xenografts. Xenogeneic demineralized bone induces bone formation in athymic rats, but not in normal rats (Aspenberg et al. 1988). On the other hand, experiments on athymic nude mice demonstrated that fresh, normal, human bone grafted intramuscularly did not survive (Hirano et al. 1991). This means that participation of humoral immune responses in xenogeneic fresh bone may be postulated.

FK is a macrolide immunosuppressant which possesses more potent immunosuppressant properties than cyclosporin, inhibiting cell-mediated and humoral immune responses. In our study, we investigated whether or not FK 506 can improve the acceptance of xenogeneic bone grafted intramuscularly into rats by suppressing the immune reaction.

We found vigorous new bone formation at 2 and 4 weeks when FK was administered. However, the previously formed bone became necrotic at 8 weeks after transplantation with small round cell infiltration around the necrotic bone which implies that the immunosuppressive effect of FK disappears before 8 weeks after transplantation.

The immunosuppression mechanism of FK is reported to consist of a strong suppression of the activation and proliferation of T cells as a result of suppression of IL 2 production (Kino et al. 1987). It has also been reported that FK inhibits differentiation and maturation of T cells in the thymus in an experimental system, leading to a decrease in the populations of CD4+ and CD5+ cells (Woo et al. 1991).

In fact, in our study the ratio of CD 4+ cells to CD 8+ cells decreased after the administration of FK. It was reported that CD5+ cells play a supportive role in the early stage of activation of T cells (Weiss and Imboden 1987). In our study as well, a decrease in the

CD5+ cell population was observed after administration of FK. This may be a result of inhibition of thymus maturation.

Humoral immunity is also said to be suppressed at a concentration of FK that suppresses cellular immunity (Lagodzinski et al. 1991). FK suppresses T cell-dependent and T cell-independent antibody production in mouse B cells (Walliser et al. 1989). It has also been reported that FK inhibits proliferation of human B cells (Morikawa et al. 1992).

In our study, the administration of FK after bone transplantation resulted in a significant decrease in the population of B cells.

We found that osteogenesis occurred as a result of suppression of not only cellular but also humoral immune reactions by FK. New bone formation occurred only when fresh bone was grafted. We therefore believe that osteogenesis was not induced by frozen grafted bone but occurred as a result of the survival of osteogenic cells in the fresh bone.

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