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Effect of Preoperative Thoracic Duct Drainage on Canine Kidney Transplantation

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Chronic drainage of the thoracic duct to the esophagus was developed in dogs, and its efficacy in immunomodulation was tested using kidney transplantation. Compared to 9.7 days in the control, the mean animal survival was prolonged to 9.9 days, 17.8 days, and 18.5 days when TDD was applied preoperatively for 3 weeks, 6 weeks, and 9 weeks, respectively. Prolongation was significant after 6 weeks. Patency of the fistula was 93.5, 80.4, and 76.1% at respective weeks. Number of peripheral T-lymphocytes determined by a new monoclonal antibody diminished after 3 weeks. All animals were in normal health, requiring no special care for fluid, electrolyte, or protein replacement.

Keywords antilymphocyte antibody, canine kidney transplantation, thoracic duct drainage

Thoracic duct drainage (TDD) has had an important role in the history of immunosuppression, which began with the demonstration that rejection is an immunologic phenomenon [1]. Strategies to weaken immune defenses for therapeutic purposes with irradiation [2] and steroids [3] culminated with the introduction of cytotoxic drugs of the 6-mercaptopurine class [4–6]. Gowans' classical studies of thoracic duct drainage in rats in 1959 [7] focused attention on the lymphocyte as a specific target for selective immunosuppression of the immune system. Attempts to reduce the lymphocyte mass with excision of lymphoid organs (spleen and thymus), antilymphocyte sera (ALS) or globulins (ALG), and total lymphoid irradiation (TLI) can be traced directly back to Gowans' work, which showed that both cellular and humoral immunity were profoundly depressed in the rat within 5 days of TDD [8]. Woodruff and Anderson [9] demonstrated an additive or synergistic effect of TDD and ALG.

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Attempts to apply TDD for clinical renal transplantation by Franksson [10] and others [11–17] were disappointing. Much later, it was shown in patients with autoimmune diseases [18] and in transplant recipients [19–21] that immunosuppression was not demonstrable until after 3 to 4 weeks of TDD instead of the 5 days in rats. Studies in large animal were hampered, as they were in humans, by the difficulty of maintaining long-term external TDD. Techniques in dogs for internal drainage of thoracic duct lymph into the esophagus also were unreliable. Canine studies were further limited by the inability to accurately monitor lymphocyte subpopulations.

We have reexamined TDD in dogs, using a modification of an old technique that allows the reliable internal drainage of thoracic duct lymph into the esophagus for more than 9 weeks. Immunosuppression was tested with renal allotransplantation. In addition, we developed a new monoclonal antibody called IYF-1, which recognizes enriched canine T-lymphocytes and allows changes to be followed in the lymphocytes of the thoracic duct lymph and peripheral blood.

Materials and Methods

Surgical Procedure

TDD. Female beagle dogs weighing 8.1–13.4 kg (Hazelton LRE, Kalamazoo, MI, USA) were anesthetized with intravenous sodium thiopental, nitrogen oxide, and halothane. To permit the easily identification of thoracic duct, the otherwise fasted dogs were given 100 mL of milk 2 h before the operation. A segment of vein into which the thoracic duct emptied was isolated after ligating the external jugular, subclavian, and inominate veins. The valves in this segment were disrupted by fine scissors to allow unimpeded flow of the lymph. The lymph was collected for 30 min. The external jugular vein segment was anastomosed to an ellipse of the cervical esophagus with interrupted 7-0 monofilament polybutester (Davis+Geck, Manati, PR) sutures, leaving the esophageal muscle layers open (Figure 1).

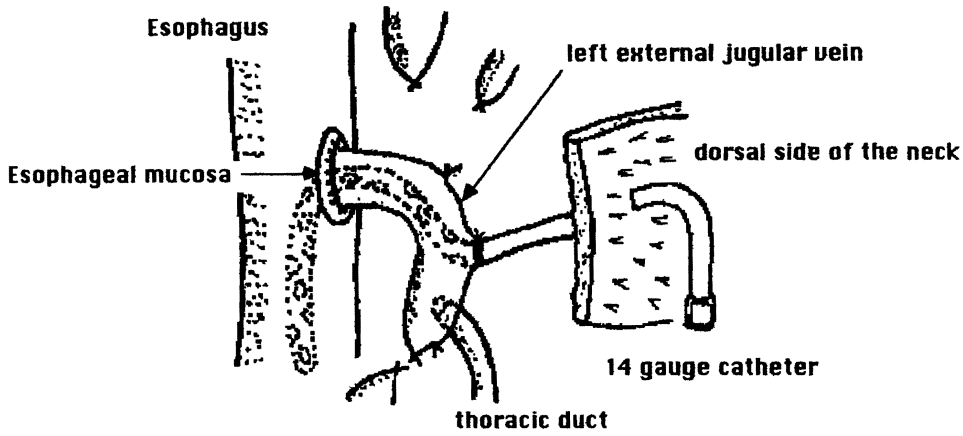

Figure 1. Isolated left external jugular vein was anastomosed to esophageal mucosa. A 14-gauge catheter on which multiple side holes were made was inserted through the brachial vein to the esophageal cavity. Its external end was placed on the dorsal side of the neck and was protected by a jacket. The catheter was flushed with 1000 units of heparin in 10 mL of saline solution several times a day.

Multiple side holes were made in a 14-gauge catheter (VYGON, East Rutherford, NJ, USA), which was inserted via a side branch of the external jugular vein segment through the anastomosis into the esophagus. The external part of the catheter was tunneled subcutaneously to the back of the neck and out through the skin. A protective jacket (Alice King Chatham Medical Arts, Los Angeles, CA, USA) was placed on the dog and the end of the catheter was seated in its pocket. The catheter was flushed several times a day with 1000 units of heparin in 10 mL of saline solution. The patency of the anastomosis to the esophagus was checked by weekly esophagoscopy performed under sodium thiopental anesthesia, using an injection of diluted methylene blue through the catheter to facilitate location of the aperture. Dogs with failed TDD were eliminated from further studies.

Kidney Transplantation. Dogs were divided into 4 groups for kidney transplantation:

A ($n = 6$): control group, not pretreated
B ($n = 6$): kidney transplant after 3 weeks TDD
C ($n = 6$): kidney transplant after 6 weeks TDD
D ($n = 6$): kidney transplant after 9 weeks TDD

In addition, 1 dog was subject to renal xenotransplantation from a pig donor, using the same operative procedures.

Anesthesia for kidney transplantation was the same as for the TDD. The jacket was taken off and the TDD catheter was removed. Kidney donors were female mongrel dogs weighing 10.4–15.4 kg. The allograft was placed into the right iliac fossa, vascularized from the iliac artery and vein, and drained with ureteroneocystostomy. Bilateral recipient nephrectomy was performed and 10 mg of furosemide was given intravenously. Cefamandol (0.5 g/day) was given IM for 10 days. No IV infusions, diuretics, or immunosuppressive agents were given postoperatively. Ad libitum diet was started the next morning. The animals were sacrificed when the serum creatinine increased to more than 10 mg/dL, or if the dogs developed disabling uremic manifestations such as vomiting and more than 20% of body weight loss. The animals were weighed once a week. Laboratory studies on days 1, 2, 3, 5, and 7, and twice a week thereafter included total serum protein, albumin, and serum creatinine. Lymphocytes were prepared on Ficoll–Hypaque gradient (Pharmacia LKB Biochemistry, Piscataway, NJ, USA) once a week and stored at –70°C until the analysis.

Immunologic Studies

Monoclonal Antibody. Balb/c mice were purchased from Harlan Sprague Dawley (Indianapolis, IN, USA) and immunized with the thymocytes of an adult female beagle dog that weighed 9.4 kg. The mice were immunized IP 4 times at 7-day intervals with 10^7 thymocytes, which were prepared on Ficoll–Hypaque gradient. Four days after the final inoculation, 10^8 of the immunized mouse spleen cells were fused with 4.6×10^7 P-3 mouse myeloma cells by polyethyleneglycol 1500 by the method of Lemke et al. [22]. After cell fusion, the cells were suspended in 200 mL of RPMI 1640 medium with 10% heat-inactivated fetal calf serum, and hypoxanthine–aminopterine–thymidine (GIBCO Laboratories, Grand Island, NY, USA). The mixture was then seeded into the cells in the presence of feeder cells obtained from Balb/c mouse spleen. After 24 h, the mixture was separated to 24-well culture plates. After cultivation for 14 days, the culture medium was replaced by a medium containing hypoxanthine–thymidine (GIBCO), and the supernatant was screened by indirect immunofluorescence for antibody activity against a variety of dog cells. The positive hybrids were cloned by limiting dilution.

The monoclonal antibody designated IYF-1, which reacted with T-cells was used for further studies.

Cell Fractionation. Macrophages were separated by differential adherence [24] from lymphocytes prepared on Ficoll–Hypaque gradient. After the separation of macrophages, T-cells and B-cells were separated by Nylon-wool column [23]. Red cells and platelets were separated by centrifugation [25].

FACS Analysis. Cells under study were treated with a saturating amount of 0.1 mL of IYF-1 for 30 min at 4°C and washed twice with phosphate-buffer solution (PBS). Those cells were then incubated with fluorescein-conjugated goat anti-mouse IgM antibody with 50% beagle dog serum-PBS for 30 min at 4°C and washed twice with PBS. The cells were fixed in 2% paraformaldehyde-PBS and samples were run on Becton–Dickinson FACScan (Mountain View, CA, USA).

Statistics

The unpaired t test and generalized Wilcoxon test were applied for statistical analysis of group means. A probability of $<.05$ was considered significant.

Results

Clinical Observations

All dogs were in normal health during TDD with no evidence of dehydration, loss of body weight, or decrease of serum total protein and albumin. Lymph flow and count from the isolated vein segment for the initial 30 min were 346.0 ± 14.4 (SE) mL and 444.9 ± 346.0/mm^3. The patency of the esophageal fistula was 93.5% in 3 weeks, 80.4% in 6 weeks, and 76.1% in 9 weeks. After kidney transplantation and the removal of the TDD catheter, the dogs remained well until the onset of rejection.

Graft and Dog Survival

Graft survival is summarized in Table 1. One dog each in groups B and C were sacrificed at postoperative days 7 and 15 with low serum creatinine levels because of intussusception. The survival of the group B animal (mean 12.0 days) was not significantly different from that in group A (9.7 days). Survival of animals in group C (17.8 days) and group D (18.5 days) was significantly prolonged.

The pig kidney transplanted to the canine recipient after 56 days TDD was hyperacutely rejected in 8 min.

Immunologic Studies

Peripheral Lymphocytes During TDD. The percentage of lymphocytes and the lymphocyte counts fell in the first week and remained depressed thereafter. The counts drifted down as long as the TDD was in place (Figure 2) from 1656.6 ± 85.4/mm^3 to 956.7 ± 83.6/mm^3 in 9 weeks. The reduction of lymphocyte count was significantly different from that of 0 weeks except at weeks 2 and 4 ($p < .01$ by unpaired t test).

Table 1
Prolongation of renal allograft survival in dogs

Group	Duration of TDD (weeks)	*n*	Survival dates[a]	Mean	*p* Value[b]
A	0	6	8, 9, 9, 9, 10, 13	9.7	—
B	3	6	7,[c] 9, 11, 11, 17, 17	12.0	NS
C	6	6	15,[c] 15, 17, 18, 18, 24	17.8	0.004
D	9	6	9, 17, 18, 22, 22, 23	18.5	0.02

[a] Dogs were sacrificed when the grafts were rejected, that is, at more than 10 mg/dL of serum creatinine, or when symptoms of severe uremic syndrome appeared, like appetite loss, vomiting, and mroe than 20% of body weight loss.
[b] By generalized Wilcoxon method.
[c] Dogs were sacrificed from intussusception with low serum creatinine level.

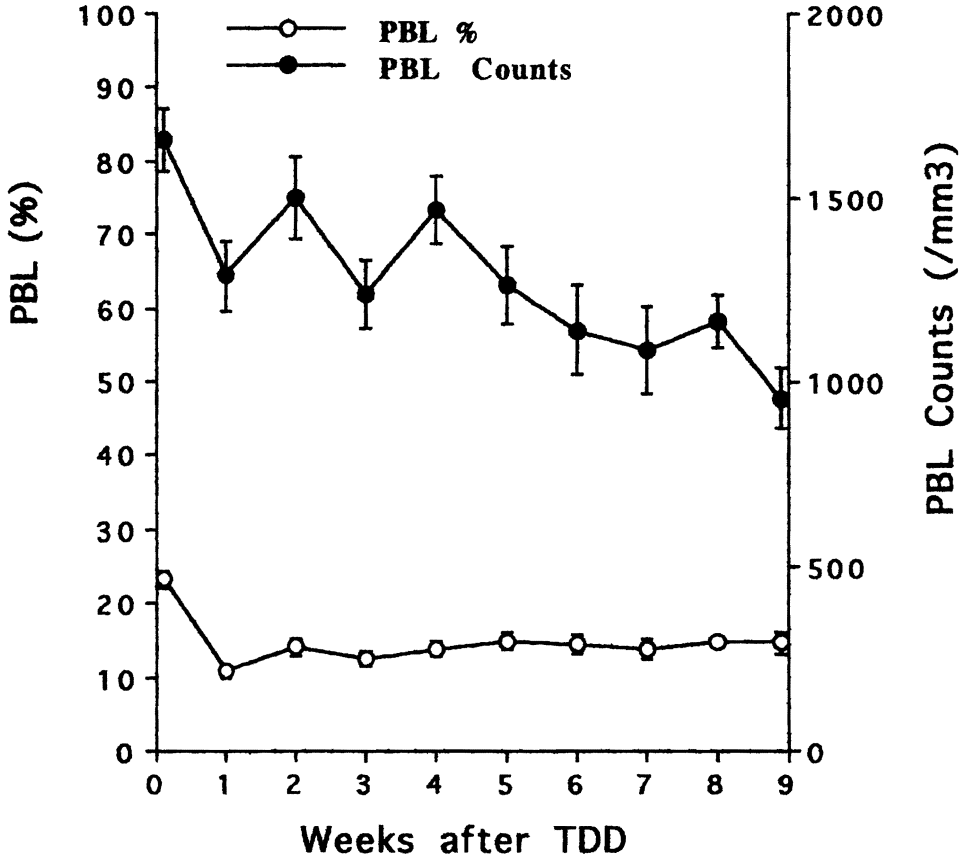

Figure 2. Changes in peripheral lymphocyte during TDD (mean ± SE). Lymphocyte counts of the 2nd and 4th weeks were not significantly different from that at 0 weeks, while others were $p < .01$ by unpaired t test.

Monoclonal Antibody and Peripheral T-cells During TDD. The immunoglobulin of subclass of the monoclonal antibody IYF-1 was IgM K light chain. All of the peripheral enriched T-cells were stained by indirect immunofluorescence, whereas B-cells, red blood cells, and platelet were not stained (Table 2). The percentage of T-cells did not show a consistent change. However, the count of T-cells went to a significantly lower level (p = .01) at all time points except week 2 (Figure 3).

Discussion

Internal TDD has been described many times since 1963 [27–32] with unsatisfactory chronic drainage, except for Kawai et al. [27] who were able to maintain patent fistulas for 6 weeks in 30% of their dogs. Our technique (Figure 1) differed from theirs mainly in the position of the catheter, which was led through the esophageal anastomosis (instead of retrograde into the duct), in the frequent flushing of the catheter, and in the placement of multiple side holes in the catheter.

The dogs were healthy after internal TDD. They did not require the fluid and protein replacement or electrolyte correction that are needed with external TDD in animals or humans [33]. The temporal development of demonstrable immunosuppression was more like that in humans than the 5-day required rats. Kidney graft survival was not significantly altered by 3 weeks of TDD but was doubled after 6 and 9 weeks. As in humans [19–21], this timing of therapeutic effect was out of phase with the T-lymphocyte count, which was depressed almost immediately after TDD and remained low thereafter.

In Gowans' original rat experiments [7] and with long-term external TDD in humans [18–21], humoral and cellular immunity were both depressed. This duality was controversial at the time of the human trials because at that time a pure cell-mediated effect was expected or reported by some investigators. The obtundation of humoral immunity in these earlier human studies was consistent with the observation that TDD appeared to favorably alter the prognosis of patients who had renal transplants against widely reacting cytotoxic antibodies [9].

Table 2
Reactivity of IYF-1 in normal beagle dog peripheral blood cells

Peripheral Cells[a]	Reaction With IYF-1 (%)
T-cells	100
B-cells	0
Macrophages	40
Red cells	0
Platelets	0

[a]By indirect immunofluorescence.

Note. Macrophages were separated by differential adherence from lymphocytes prepared on Ficoll–Hypaque gradient. After the separation of macrophages, T-cells and B-cells were separated by Nylon-wool column. Red cells and platelets were separated by centrifugation.

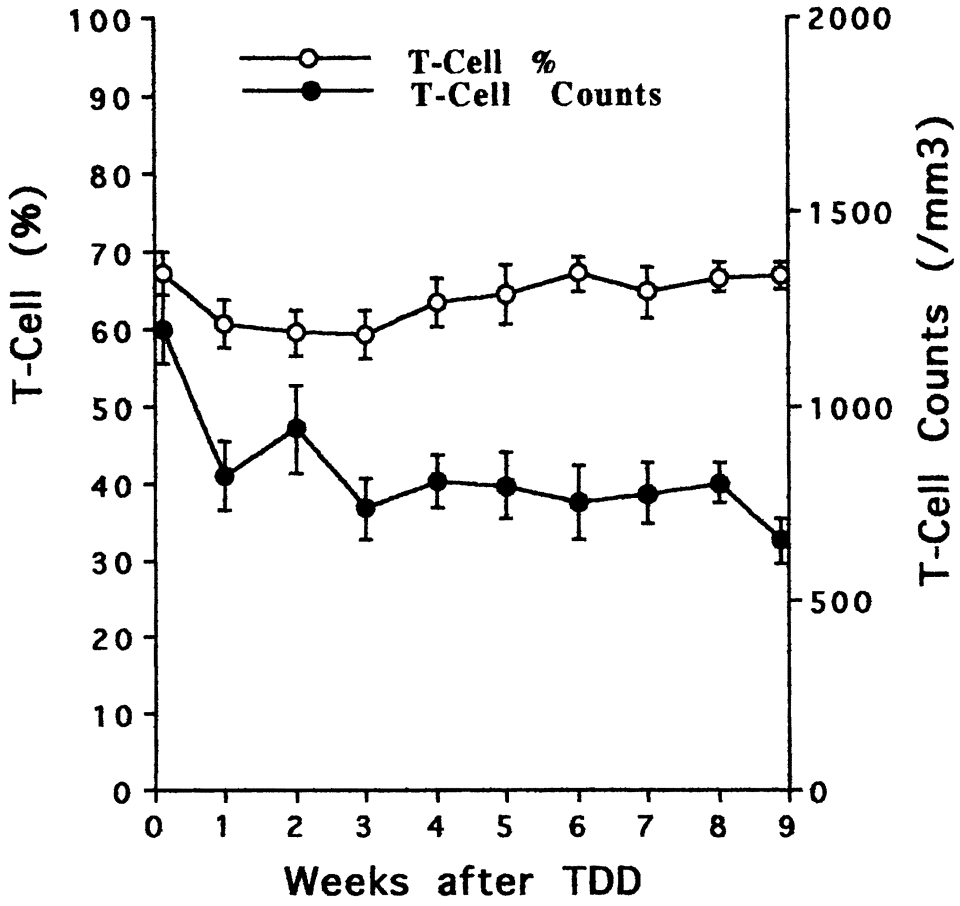

Figure 3. Changes in peripheral T-cells during TDD (mean ± SE). Population of T-cells was measured by indirect fluorescent using monoclonal antibody, IYF-1. T-cell count of 2nd week was not significantly different from that at 0 weeks, while others were $p < .01$ by unpaired t test.

The present experiments were not designed to test if T-lymphocyte depletion can affect humoral reactivity, except for one experiment of pig to dog renal heterotransplantation. The heterograft was hyperacutely rejected in spite of 56 days pretreatment with TDD. It is possible that a secondary loss of B-lymphocyte and NK cell function will require much longer periods of T-cell depletion.

Examination of such questions is increasingly possible because of the monoclonal antibodies made possible by the somatic cell hybridization techniques of Kohler and Milstein [34]. However, although dogs are frequently used in transplant studies, very few monoclonal antibodies are available for characterization of dog lymphocytes [35–40]. Our monoclonal antibody, IYF-1, was raised against beagle dog thymocytes and had an reactivity to 100% of peripheral enriched T-cells. There is the possibility that IYF-1 will have therapeutic effect in dogs similar to that of OKT-3 in humans. Further studies to test this hypothesis are planned.

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