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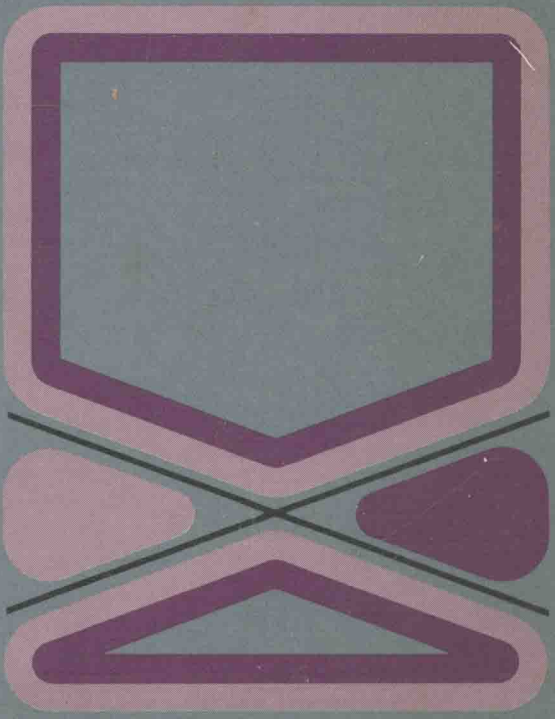

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Editors:
J.L. Touraine
J. Traeger
H. Bétuel
J. Brochier
J.M. Dubernard
J.P. Revillard
R. Triau

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IMMUNOSUPPRESSION

POSTER SESSION

Part 1

THORACIC DUCT DRAINAGE (TDD) IN RENAL TRANSPLANTATION; A CRITICAL REVIEW

C. Franksson and G. Lundgren

Departments of Surgery and Transplantation Surgery, Huddinge Hospital, 141 86 Huddinge, Stockholm, Sweden

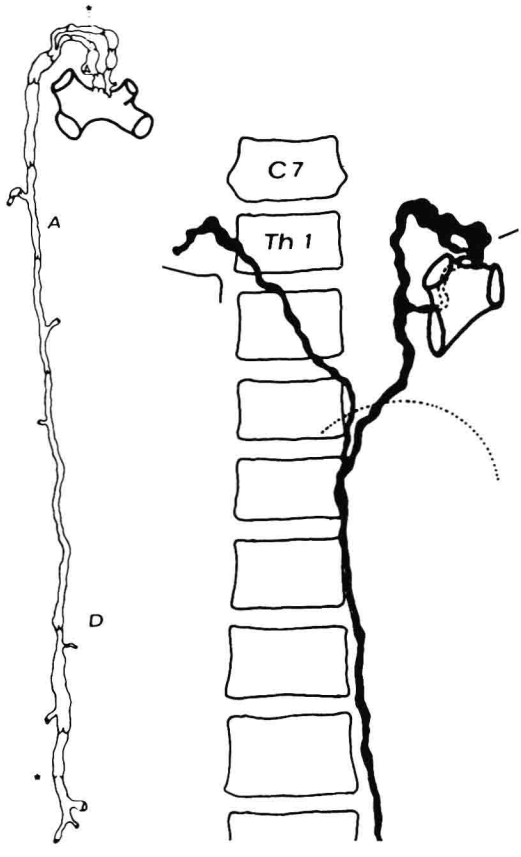
The fundamental importance of the lymphocytes for the immunological capacity and for the rejection of allografted tissue was elucidated by the pioneering work of Medawer (1) and Mitchinson (2). A prerequisite for successful allogeneic transplantation in man has been and still is that the activity of the lymphocytes can be suppressed by e.g. irradiation, cytotoxic drugs, or by reducing the size of the lymphocytic pool. All these methods have been used.

Lymphocyte depletion by TDD was first tested for the treatment of lymphatic leukemia (3,4) and then in renal transplantation (5,6). In rats, it was demonstrated that a chronic thoracic duct fistula generated a peripheral lymphocytopenia and central lymphoid atrophy (7) which ablated or markedly inhibited the primary humoral antibody response to antigens such as sheep erythrocytes and tetanus toxoid, while the secondary humoral immune response to these antigens was relatively unchanged (8,9).

Formation, transport, and composition of lymph

The lymphocytes, the main cell of the lymph, are formed in the bone marrow and maturate in the lymphoid organs, including thymus, spleen and the lymph nodes. The lymphocytes are transported to the blood mainly through the lymph vessels where they are accessible for interventions such as drainage and irradiation. The lymph also contains large amounts of proteins, including immunoglobulins, which may be of importance in the process of rejection.

The lymph vessels from the lower extremities and the abdominal organs join just below the diaphragm in the cisterna chyli. The thoracic duct, which is the largest lymph vessel in the body, arises from the cisterna chyli and ascends through the thorax on the anterior aspect of the vertebral bodies. At the level of the seventh thoracic vertebra it curves over to the left and joins the venous system at the junction of the left subclavian vein and the left internal jugular vein. The last part of the thoracic duct is often split into two to five branches. The lymph from the upper lobe of the left lung also enters the thoracic duct. The thoracic duct contains valves which are most numerous in the cervical portion which renders cannulation more difficult (Figure 1). In four per cent of the cases the upper part of the thoracic duct is connected with the right lymphatic duct (Figure 2) (10). The lymph vessels from the right upper part of the body, the right arm, the heart, the right lung and the lower lobe of the left lung join and form the right lymphatic duct which enters the venous system at the venous junction on the right side.



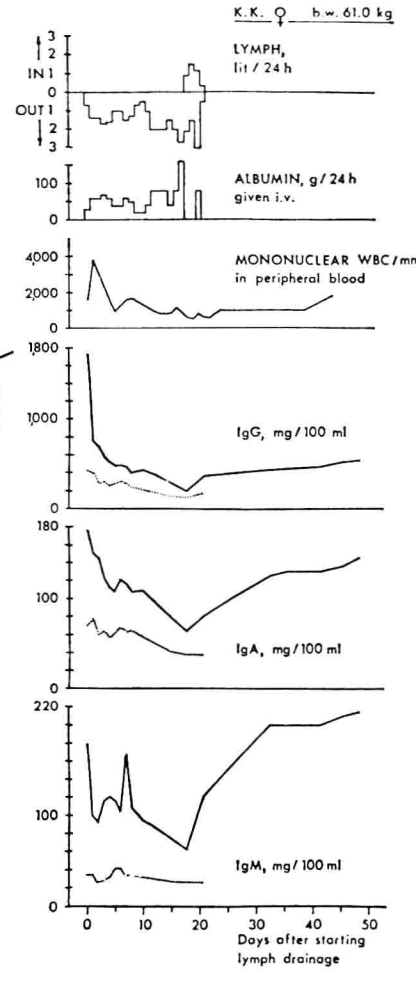


Figure 1 Figure 2 Figure 3

Figure 1. Drawing of an autopsy specimen of the thoracic duct showing the distribution of valves and two openings to the venous system.
Figure 2. Drawing of a lymphangiogram of the thoracic duct in an cadaver. The duct is divided into two branches, one terminating in the left and the other in the right brachiocephalic vein.
Figure 3. Changes observed in the peripheral blood and the thoracic duct lymph (lower line) during TDD and retransfusion of albumin and lymph.

The protein content of the peripheral lymph is low (20 g/l). The intestinal lymph is richer in proteins (30-50 g/l) and the liver lymph has about the same protein content as blood (60 g/l). The liver lymph makes up about half of the lymph in the thoracic duct where the protein content is about 40 g/l). A daily amount of about 1-2 liters of lymph containing 40-80 g protein enters the venous system via the thoracic duct. The thoracic duct is also a main transport route from the intestines to the blood for lipids with long carbon chains (>10 C-atoms) and vitamin A. After a fatty meal the lymph may contain 1-2% lipids and looks like milk. Bacteriae and malignant cells are also transported via the lymph (4). The lymph contains the same type of cells as the blood but in different proportions (Table 1). The dominating cell is the small lymphocyte, which enters the lymph from the lymph nodes and thymus. Polynuclear leucocytes are sparse and erythrocytes are normally few. During, and some days after surgical operations, the number of erythrocytes increases and the lymph which normally has a slight yellow colour may become red as blood. Platelets are missing in normal lymph but appear in big numbers when the lymph gets bloody which increases the risk for clotting (4).

TABLE 1. Cell counts in thoracic duct lymph

Lymphocytes	1.0-7.0 x 10^9/l
Polynuclear leukocytes	0.04-0.5 x 10^9/l
Erythrocytes	0.2-0.5 x 10^9/l

Cannulation of the cervical part of the thoracic duct

The thoracic duct enters the venous system only 1-2 cm under the skin. This area can be explored in local or general anesthesia. A polyethylene catheter with a diameter of about 2 mm is passed into the duct through a small incision and is fixed with a ligature. There are numerous valves in this part of the thoracic duct which renders the introduction of the catheter more difficult (Figure 1). However, it is usually possible to pass some of these valves after which the catheter can be anchored. In connection with surgical operations the lymph often gets mixed with blood and may easily clot. The risk for clotting can be reduced by the use of a double lumen catheter permitting a continuous infusion of small amounts of heparin. Sometimes, however, the catheter gets occluded which necessitates a re-operation and changing of the catheter. This procedure is usually easier than expected.

The drained lymph volume is about 1-3 l/day in a normally hydrated patient which means a substantial loss of fluid, electrolytes, carbohydrates, lipids, proteins etc. A patient who feeds himself orally can easily replace all losses except the proteins. The lymph contains about 40 g protein/l which means a daily loss of 40-120 g. Sooner or later the proteins must be replaced intravenously, either by the drained lymph after removal of unwanted components, or by plasma or commercially available serum albumin. If serum albumin is used the concentration of endogenous proteins, including the immunoglobulins, declines. It is more economical to retransfuse the patient's own lymph, in which case no reduction of the immunoglobulins occurs (Figure 3).

When a sufficient amount of lymph has been drained, the flow is slowed down by a gradual elevation of the tip of the catheter, Usually the flow stops completely after a few days. The catheter is then occluded and left in place for about a week and can then be removed without risk of fistula formation. By these measures the lymph pressure in the thoracic duct increases and dilated lymph vessels can be seen in the abdomen at laparotomy. However, the lymph will find new routes to the venous system within a week or so after which the lymph stasis disappears. Exceptional cases have been reported where the distension of the lymph vessels has caused severe abdominal pains necessitating recannulation of the fistula or implantation of the thoracic duct into a vein (11).

Complications of cannulation. The reported complications are rare and mild (12). Leakage of lymph at the cannulation site may occur creating a subcutaneous chyloma. The leakage usually stops after removal of the catheter and application of a compression bandage. Rarely is it necessary to re-explore the thoracic duct and ligate it below the leakage.

Lymph leakage to the pleura sometimes occurs and most often on the left side. Usually the volumes are small and are spontaneously resorbed. In exceptional cases the volumes are bigger and may necessitate repeated tapping of the pleural cavity. Infection at the site of cannulation is sometimes seen. It usually heals after opening of the wound, removal of the catheter and administration of adequate antibiotics. Actue pancreatitis (11), temporary pareses of the left vocal cord (13) and Horners syndrome (14) have also been reported.

Effects of lymph drainage

Drainage of large amounts of lymph causes several changes. Some of these can be detected only by laboratory methods, while others are observed clinically.

Laboratory findings. Figure 3 illustrates some characteristic findings in the peripheral blood and the lymph during and after discontinuation of lymph drainage. The patient was a 29-year-old woman. The protein losses were replaced by commercially available human serum albumin during the first 18 days. During this time both the absolute number of lymphocytes in the peripheral blood and the amount of immunoglobulins decreased. When cell free lymph was re-transfused, and when somewhat later the drainage was stopped, the immunoglobulins and the lymphocyte number in the blood started to rise. The reduction of the immunoglobulins is fully developed after about one week, but an effect on the lymphocyte number in blood often requires considerably longer drainage. The profound reduction of the immunoglobulins is avoided if cell free lymph is re-transfused (15, 16). Other investigations (14, 17), however, report a significant reduction of the IgG concentration after one week in spite of re-transfusion of lymph plasma. The IgM concentration, however, remained unchanged.

The dominating cell in the lymph is the small lymphocyte. A chronic thoracic duct fistula causes peripheral lymphocytopenia and atrophy of the peripheral lymphoid organs (3, 4, 7). This has been confirmed in a number of investigations. However, the effect on the lymphocyte number in peripheral blood occurs later and is of less magnitude than should be expected considering the vast amounts of lymphocytes that are eliminated.

The concentration of lymphocytes in the lymph is highest during the first week and numbers of 10×10^9 drained lymphocytes per 24 hours have been reported (18,19). This means that the number of lymphocytes drained per 24 hours equals the number of lymphocytes in the whole blood volume. Still it often takes 2-4 weeks until a reduction of the lymphocyte number in peripheral blood is obtained (18, 19, 20) and some authors have reported an unchanged number of lymphocytes in the blood in spite of further prolonged drainage (14, 21). In adult humans 60-75% of the thoracic duct cells are T-cells. Prolonged drainage should then cause a selective depletion of T-cells in the peripheral blood. This has also been reported by some authors (17, 18, 19, 20) while it has not been a consistent finding according to other authors (14, 21).

Clinical observations. The reported findings (8, 9) that the primary humoral antibody response is markedly inhibited by lymph drainage in rats have also been verified in man in a number of investigations (14, 17, 22, 23). These authors also demonstrated a profound effect of thoracic duct drainage on delayed hypersensitivity skin reactions to a variety of antigens. In one study the reactivity against intradermal antigens such as mumps and candida disappeared after 25 days drainage (17) while in another investigation positive skin tests turned negative after 3 weeks drainage in 8 of 9 patients (14).

Thoracic duct drainage has been used to treat various acute rheumatic disorders and other types of autoimmune diseases in man. Usually there is a good effect on the clinical symptoms which are either relieved or completely disappear during the period of drainage (24, 25, 17, 26). The same is true for myasthenia gravis where a specific symptom promoting factor, an autoantibody (IgG) against the cholinergic receptors of the muscle cells, has been demonstrated in the lymph (27, 28).

The most important clinical application of the thoracic duct drainage is, however, in the context of organ transplantation. The method was introduced as a adjunct to chemical immunosuppression in renal transplanttion (5) but has also been tried in liver (29) and pancreatic transplantation (21, 30). It has also been used in bone marrow recipients in order to moderate severe graft-versus-host disease (31).

Renal transplantation

Thoracic duct drainage in renal transplantation has now been tried by several groups under various conditions, and the experience that has emerged from these studies has elucidated the indications for TDD and how it should be used.

Thoracic duct drainage has been tried alone without any other immunosuppressive methods in a few cases (15, 32). Two of our patients did not take any chemical immunosuppressive drugs for 49 and 102 days, respectively, at which time they started to show signs of rejection. The first patient irreversibly rejected his kidney while the other patient, after addition of chemical immunosuppression, lived for 11 years with a well functioning graft. It seems obvious that thoracic duct drainage alone is insufficient for immunosuppression in organ transplantation and should be combined with chemical immunosuppression. Most groups have combined TDD with azathioprine and steroids. A beneficial effect of ALG in addition to TDD has been demonstrated (11, 32). In our own series the best results were obtained with a combination of TDD + ALG + thymectomy + azathioprine + steroids. This may indicate that a simultaneous attack on T-cells at various levels is most effective.

In the early clinical cases the patients were drained for relatively short periods since an unexpected negative effect of the profound lymphocyte depletion could not be excluded (33). However, with growing experience the period of drainage was prolonged and favourable results upon graft survival were reported by several groups. It was thus shown that drainage for more than 28-30 days significantly improved the results after cadaveric kidney transplantation (32, 34).

Theoretical considerations would favour lymph drainage as pretreatment to transplantation. Most groups have utilized TDD in this way. Some investigators (16, 19) have required a reduction of skin tests or in vitro tests as a sign of adequate depression of the patients immunological status before the patient can be placed on "transplant alert", while others have set a certain time limit for the drainage (11, 20, 34). Touraine and co-workers (11) considered 2-3 weeks drainage efficient if the lymphocyte depletion reached 20×10^9 cells. This number, however, is small in relation to the depletion reported by many other groups.

When cadaveric kidneys are used, pretreatment is difficult to arrange since the day of transplantation cannot be forseen. Therefore, in Stockholm the lymph drainage is usually started 1-3 days after renal transplantation. Chemical immunosuppression has been trusted to sufficiently depress the immunological system during the first few weeks. The long-term results do not seem to be adversally affected by this policy and are the same as reported by other groups. Starzl and co-workers (34) were, however, unable to achieve as good results with post-transplantation drainage as compared to pretreatment drainage. The difference in graft survival between the groups was however small.

Betuel and co-workers (35) studied the effect of TDD on patient groups receiving different numbers of blood transfusions before transplantation. It was especially noticeable that a graft survival of 86% at 18 months was obtained in the group with no blood transfusions. Many studies have shown this group to have an extremely low survival rate.

Seven groups (11, 16, 19, 20, 32, 35, 36) have extensive experience of thoracic duct drainage and their results concerning graft survival are summarized in Table 2. A significant improvement on graft survival after cadaveric kidney transplantation was usually demonstrated in the drained group compared to non-drained controls (11, 18, 20, 32) while no significant effect of thoracic duct drainage could be demonstrated when living related donors were used (11, 32, 36).

TABLE 2. Graft survival (%) of cadaveric kidneys (CD) and living related donor kidneys (LD) in patients treated with TDD

	CD kidneys, yrs					LD kidneys, yrs				
	1	2	3	5	10	1	2	3	5	10
Touraine et al. 1977 (11)	70	70	60	50		81	71	64	62	
Betuel et al, 1982 (35)		71								
Tilney 1977 (36)						88	78	70	70	
Kaplan 1979 (19)	79									
Richie et al. 1980 (16)	72	69	62	62						
Fish et al. 1981 (20)	87		80	80						
Starzl et al. 1981 (34)*	72									
Franksson et al. 1983 **	83	83	83	60	53	82	78	78	70	48

* TDD>28 days
** TDD>30 days

Starzl and co-workers (34) achieved an improvement of graft survival in the lymph drained group but late graft failures eliminated the difference in relation to the controls. A difference persisting for 5 years, however, has been reported by other groups (11, 18, 20). In our own material the graft survival is significantly better in the lymph drained group even after 10 years.

The indications for thoracic duct drainage have been modified during recent years, and is nowadays mostly provided in cases where a complicated immunological course is expected. Thus, thoracic duct drainage has been used to ensure a satisfactory survival of badly matched kidneys (18, 19, 20) and in patients who have rapidly rejected one or more grafts as a sign of high immunological responsiveness (16, 21). TDD is also used in recipients of living related donor kidneys but only in cases with a high relative response in the MLC reaction (21).

DISCUSSION

The mechanism behind the favourable effect of prolonged thoracic duct drainage on graft survival is unclear. The most immediate explanation would be T-cell depletion. However, it has been suggested that the removal of factors in the cell free lymph might be responsible (22). Other investigators (16, 20) who centrifuged and re-transfused the cell free lymph demonstrated that lymphocyte depletion prolonged allograft survival in the presence of normal and unchanged serum immunoglobulin levels. A considerable reduction of the IgG level may occur, however, in spite of re-transfusion of the lymph plasma (14, 17). It is of special interest in this context that thoracic duct drainage has made renal transplantation possible in patients with broadly reacting antibodies and strongly positive crossmatches against the lymphocytes from the kidney donor (14, 16). In spite of a strong positive crossmatch with a current recipient serum in one study (16) 3 of 4 patients had a functioning graft at 10 months, 6 years and 6 years, respectively. One patient died with a functioning graft at 6 weeks. In another study (14) 6 patients with broadly reacting warm anti T-cell antibodies escaped hyperacute rejection, but all were slowly rejecting within 13 months. Hyperacute rejection was avoided in spite of re-transfusion of the cell free lymph.

As mentioned above, a reduction of the number of lymphocytes and T-cells in the peripheral blood is not a constant finding in spite of prolonged lymph drainage. Despite unchanged number, several authors have demonstrated a profound inhibiton of the ability of peripheral blood lymphocytes to react against various mitogens and especially against allogeneic lymphocytes in mixed lymphocyte cultures. The inhibitory effects were recorded after 2-4 weeks drainage (11, 18, 21). Since these reactions are T-cell dependent, the results point to an inhibition of T-cell functions. The discrepancy between the absence of a quantitative effect on the T-cells in the blood and the profound inhibition of specific T-cell functions would imply that thoracic duct drainage specifically eliminates a subpopulation of T-cells which are responsible for the proliferative reaction in MLC and the response to mitogens (11, 18, 21). These sepculations are strongly supported by a recent study applying the technology of monoclonal antibodies (37). After 3-5 weeks lymph drainage the relative number of OKT4 positive cells (T helper cells) decreased significantly.