

# Acquired Lymphedema: An Urgent Need for Adequate Animal Models

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## Abstract

**In cancer patients, the removal of tumor-draining lymph nodes during tumor resection can lead to acquired lymphedema. This disease, which is characterized by tissue swelling and increased risk of infection due to restricted lymph flow, lacks an effective treatment. Limitations to the design and conduct of randomized trials to date have limited the evaluation of proposed surgical techniques. As a result, animal models have provided an important research base. This review summarizes work in canine, rabbit, and rodent models of acquired lymphedema, focusing on arising limitations and potential applications. [Cancer Res 2008;68(2):343–5]**

## Introduction

Lymphatic transport results from a delicate balance between peripheral lymph production and lymph drainage to the central veins. Lymphatic collectors in humans are provided with a continuous layer of smooth muscle. This permits a serial contraction of the vessel segments between the valves, resulting in unidirectional lymph flow. But only the additional synergetic action of muscle contractions in the extremities, breathing movements, and passive body positioning creates an effective lymph drainage dynamic. An unimpaired flow through the lymph nodes is also essential in preventing the peripheral accumulation of intercellular fluid, macromolecular proteins, and cell detritus as seen in lymphedema.

In developed countries, acquired lymphedema mainly occurs as a corollary of oncological surgery. Particularly surgery for breast, vulva and cervical cancers, and also for melanoma of the extremities, has been associated with high rates of lymphedema. The necessary removal of regional lymph nodes to improve survival rates causes a disruption of lymph drainage. When compensatory mechanisms fail in transporting the complete lymphatic load, lymphedema occurs. Generally, patients, who undergo more extensive surgery have many lymph nodes removed, need postoperative chemotherapy, and/or are submitted to radiotherapy after surgery are more likely to develop lymphedema (1). However, the time span between cancer operations and onset of lymphedema can vary from some weeks to several decades. This renders extrapolation of incidence data very arduous because of divergent follow-up concepts. Nevertheless, the cross analysis of the incidence rates reveals the magnitude of the problem. Breast cancer-related lymphedema of the arm has an overall incidence of around 30% in women treated (2). Currently, there are about 147,000 breast

cancer survivors living in Europe, and 2 million in the U.S.A. Additionally, lymphedema of the lower extremities after cervical and vulval cancers affects 20% to 49% of the women postoperatively (2). When malignant melanoma has to include ilioinguinal lymph node dissection, around 20% of the patients develop lymphedema (2).

The therapy of lymphedema is based on lifelong physiotherapy. However, a great number of patients do not have access to qualified professionals and long term compliance is often poor. In an attempt to preclude the onset of this disease, surgeons increased the efforts of prevention. The routine execution of sentinel node biopsy contributed greatly to the reduction of lymphedema, but in a significant number of patients radical lymph node dissection and/or radiation are still needed. The recent practice of Axillary Reverse Mapping during breast cancer surgery is another example of the surgeons' concern about prevention (3). Interestingly, breast cancer excision alone without radiation or harvesting of any axillary lymph nodes is shown to have a 4% incidence of lymphedema of the arm (4). This suggests an involvement of mechanisms other than direct intervention on the lymph nodes in the generation of chronic postoperative lymph congestion.

Additionally, efforts have been made to find new therapies for this condition. A large number of surgical procedures have been proposed over the years, but none presented groundbreaking long term results. In the search for evidence-based therapies, many researchers have tried to create animal models for acquired lymphedema, but most of them were unsuccessful.

## Beginnings with Canine Models

As early as 1888, Delius (for review see ref. 5) tried to induce lymphedema in dogs by ligating the lymphatic trunks near the saphenous vein in the hind limb, but no postoperative swelling occurred. Drinker (as reviewed in refs. 5, 6) included meticulous circumferential measurements of the limbs of the animals in his methodology. He was nevertheless unsuccessful, although he thoroughly excised all lymph nodes and lymphatics of the groin. Therefore, he additionally injected sclerosating agents in the central lymphatic trunks. But it was only after repeatedly inoculating streptococci in the hind limbs of the dogs that he was able to produce stable lymphedema.

Olszewski and colleagues (for review see refs. 5, 6) left a gap in the surgical wound to impeach the great impetus for restoration of s.c. lymph vessels. In their series of 23 dogs, they transected the main lymphatics of the hind limb and then removed a circular strip of skin, s.c. tissue, fascia, and periosteum. The tissue gap was left to heal through secondary granulation. One-third of the animals developed permanent lymphedema. This technique was later improved by completely excising the lymphatic chains of the thigh during a second operation, which resulted in a lymphedema frequency of 66.7%. This model was used to study lymphaticovenous microanastomoses (as reviewed in ref. 6). Researchers showed that postoperative pressure gradients between the lymph and

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venous blood were inversed when lymphedema tended to resolve, thus causing lymph reflux and thrombosis of the anastomosis.

Das and colleagues (for review see ref. 6) extensively irradiated the groin of 15 dogs preoperatively with a single dose of 15 Gray (Gy). An extensive excision of all connective tissue, muscle fascia, and deep lymphatics was then performed. All 10 surviving dogs developed lymphedema persisting for at least 12 months. Based on this model, other groups were able to show that lymphedematous limbs have a diminished activity of various tissue proteinases including collagenase, thus providing the first insights on the mechanisms of tissue alteration and skin fibrosis occurring in this disease (7).

Chen et al. (for review see ref. 6) irradiated dogs in the knee with a single dose of 12 Gy. A surgical excision of a circumferential strip of skin and s.c. tissue followed. Innovatively, the skin edges were sewed to the underlying muscle fascia and left to heal. A second operation was then undertaken to remove all the deep lymphatics and lymph nodes. Twelve of seventeen dogs showed a permanent increase in limb volume. Using its own model, this group ascertained the results of autologous microsurgical lymph node transplantation to the knee. The lymph nodes were transplanted, and a microanastomosis of the artery and vein performed in all of them. Additionally, in one group, a microanastomosis of the afferent and efferent lymphatics to the node was carried out. Surprisingly, all dogs showed improvement of their lymphedema irrespective of the group, suggesting that lympho-lymphatic anastomosis in microsurgical lymph node transplantation has little clinical relevance. Regrettably, no dog presented a complete resolution of the disease (6).

Despite the variety of methods, none of the canine models for lymphedema reached great acceptance. The complexity of the procedures and relatively long latency time before chronic lymphedema was established were barriers to their success.

### Lagomorpha for Lymphedema

Casley-Smith introduced the concept of using a rabbit ear model for lymphedema (for review see ref. 6). This model is anatomically advantageous because the main blood vessels and principal lymphatics are located in the external base of the ear rendering surgical dissection easier. Additionally, deep lymphatic structures are absent, and the underlying cartilage provides mechanical support to the necessary surgical resection of a circular tissue flap (8). Rabbits are more affordable, and operating ears is not as time consuming as operating limbs. This ear model proved to be very effective in generating chronic lymphedema. Huang and coworkers (as reviewed in ref. 6) reported a stable lymphedema >6 months in 47 of 50 operated rabbits. Szuba (9) and Rockson (10) used it to show the positive effect of gene therapy in lymphedema management. They administered a viral vector with the gene for vascular endothelial growth factor-C (VEGF-C) s.c. in the swollen ears. VEGF-C is a mitogenic molecule that specifically governs the development and growth of lymphatics. All rabbits with lymphedema receiving VEGF-C gene showed a histologically confirmed lymphangiogenesis and a decreased dermal fibrosis in comparison with the control group.

Nevertheless, Fu and colleagues (11) criticized the necessity of perichondrium removal. All their rabbits showed a necrosed auricular cartilage after the intervention. In their following series, they spared the perichondrium and were still able to induce lymphedema in most of the animals.

Youn and coworkers (12) interestingly reported that in young rabbits, no single animal developed ear lymphedema after this standardized operation. It was only effective when 4-year-old rabbits were used. The reasons for this age dependence are unknown.

Due to these difficulties, rabbit ear models lost popularity, leaving researchers with the task of looking for alternatives.

### Benefits of Rodent Models

Although inexpensive and easily accessible, rodent models were only reported in the 1980s. Wang and coworkers (for review see ref. 6) tried to generate lymphedema in the hind limb of rats by removing a circular strip of skin and s.c. tissue and resecting the underlying lymphatics. The survivors developed an unexpected lymphedema of both hind extremities, rendering volumetric controls impossible.

It was not until this surgery was combined with preoperative radiation that lymphedema of the hind limb could be successfully induced (for review see ref. 5). Curiously, radiation alone was insufficient to induce any swelling. Only rats given a single dose of 45 Gy combined with a limb operation developed a sustained lymphedema. Lee-Donaldson and colleagues (as reviewed in ref. 6) later showed that preoperative radiation was as effective as postoperative radiation in creating chronic lymphedema, as long as surgery and radiation were combined at some point of the process.

Slavin and coworkers (for review see refs. 5, 6) introduced a new mouse model. They performed a circumferential incision at the base of the mouse tail, but instead of resecting a skin flap, they retracted the skin to create a 3-mm gap. This hiatus was then cauterized along with the deep lymphatics. All mice submitted to the procedure developed a sustained lymphedema of the tail. This model has gained great popularity due to the ease of the procedure and its effectiveness. Its invention brought about a real boost of information about molecular aspects of lymphangiogenesis. Due to this model, the beneficial aspects of VEGF-C on lymph flow were confirmed (13). Other lymphedema protective factors such as matrix metalloproteinase-9 (14) or hepatocyte growth factor (15) could be accredited. It was shown that genes responsible for inflammation and oxidative stress were up-regulated in acquired lymphedema of the tail (16), thus providing new insights on the molecular keys of this disease.

### Conclusions and Future Directions

Gaining a historical perspective about research on acquired lymphedema reveals that groundbreaking findings arose from the generation of appropriate models. Nevertheless, animal models are not universal. Whereas the mouse tail model seems appropriate for molecular studies, its use in the development of curative surgical techniques, for example, has obvious disadvantages. Physiologic studies need to take into account the anatomic differences of each species. For instance, lymph vessel walls of dogs and rabbits display scattered smooth muscle, whereas rodents surprisingly present muscular patterns that are much more similar to human type. A comparison of some model characteristics is summarized in Table I.

Anatomic structures associated with immunity are highly adaptive. Therefore, generating lymphedema in animals can be extremely difficult. On the other hand, the great regenerative capacity of lymphatics has proved very rewarding in oncological reconstructive interventions. However, the current operative interventions for acquired lymphedema could not establish themselves as part of the

**Table 1.** Merits and disadvantages of present lymphedema models

Model	Canine hind limb	Rabbit ear	Rodent	
			Limb	Tail
Lymphedema-inducing operative technique	Complex	Accessible	Complex	Very easy
Rate of operative success in inducing lymphedema	66.6 (5, 6)–70.6% (6)	94% (6)	100% (5)	100% (5, 6)
Type of lymph nodes	Normal type	Normal type	Blood type (20)	Blood type (20)
Type of lymph vessels	Low muscle	Low muscle	High muscular (20)	
Adequacy for development of new curative surgery	Tissue gap renders transplants difficult Epifascial and subfascial collectors in close anatomic proximity and in low number compared with humans (20)	Tissue gap renders transplants difficult Epifascial and subfascial collectors in close anatomic proximity and in low number compared with humans (20)	Tissue gap renders transplants difficult Epifascial and subfascial collectors in close anatomic proximity and in low number compared with humans (20)	Confined operative field Confined operative field

usual therapy, although some interventions apparently allow physiotherapy to be discontinued in a significant number of patients (17). The necessity of ameliorating curative operative techniques depends on the development of new animal models, as the ones available seem relatively inappropriate for surgical issues.

Last but not least, in developing countries, surgical interventions for postcancer lymphedema are already being extrapolated to treat filariasis-associated lymphedema with relative success (18). The

latter is a pandemic with >40 million people affected worldwide (19). That is why a new impulse to research lymphedema is urgent and necessary.

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