

Cystic Hygroma Reconsidered: Hamartoma or Neoplasm? Primary Culture of an Endothelial Cell Line from a Massive Cervicomedial Hygroma with Bony Lymphangiomas

Carol A. Bowman, Marlys H. Witte, Charles L. Witte, Dennis L. Way, Raymond B. Nagle, Jack G. Copeland, Charles C. Daschbach

Departments of Surgery and Pathology, University of Arizona, Tucson, and Department of Medicine, St. Joseph's Hospital, Phoenix, Arizona

Abstract:

A young woman presented with massive enlargement of a giant cervicomedial cystic hygroma, which communicated in part with the thoracic duct and was associated with generalized bony lymphangiomas. Modern imaging and sophisticated intraoperative physiologic monitoring made one-stage resection feasible. Tissue culture of explants of the hygroma yielded a primary endothelial cell line still surviving after 18 months, which, like the cyst-lining endothelium in the original resected specimen, reacted positively for Factor VIII-associated antigen. These findings, in conjunction with the histologic picture, support the notion that cystic hygroma represents an expanding proliferating endothelial growth process and not simply a sequestered lymphatic receptacle.

Cystic hygroma is an uncommon benign outgrowth of the lymphatic system composed of irregular cystic spaces lined by vascular endothelium. A congenital etiology is generally accepted, and most lesions are indeed first detected in infants or young children. On the other hand, initial presentation or impressive enlargement may occur during adulthood. Although non-specific factors such as trauma, infection or spontaneous hemorrhage have been implicated in rapid enlargement, it remains unclear whether these lymphatic anomalies are hamartomas (i.e. malformations of developing lymphatic trunks or sacs) or rather neoplasms capable of endothelial proliferation and even "metastasis" to distant sites.

This case report describes an adult patient with a giant cervicomedial

cystic hygroma in whom massive enlargement during adulthood, associated osseous lymphangiomas, and proliferation of a primary endothelial cell line in tissue culture raise fundamental questions about the origin, nature, and growth potential of these lesions.

Case Report

A 29 year old American Indian woman was referred to Arizona Health Sciences Center for evaluation and treatment of a massive cervicomedial cystic hygroma presenting as a soft, painless, enlarging left supraclavicular mass (Fig. 1A) for the past two years. She complained of recent onset of dysphagia characterized by a sensation of substernal "hanging up" of solid foods, mild dyspnea on exertion and 10 pound weight loss over the previous 4 months. At 15 years of age, she had undergone excision of a small cyst in the right neck (histologically confirmed as cystic hygroma), and two years later a chest radiograph suggested mild prominence in the right paratracheal area. Chest radiography now revealed a huge anterior mediastinal mass (Fig. 2A,B). After incisional biopsy of the protruding left supraclavicular mass the typical histologic picture of cystic hygroma was affirmed.

Physical examination revealed a nontender, fluctuant 15 x 15 cm left supraclavicular mass that partially transilluminated and deviated the trachea to the right (Fig. 1A). Valsalva maneuver (Fig. 1B) and the head-down position (Fig. 1C) exaggerated the cervical bulge, and the overlying external jugular vein became intensely engorged. Dullness to percussion and diminished breath sounds were noted over the anterior chest bilaterally.

Laboratory examination including hemogram, urinalysis, renal and liver battery were

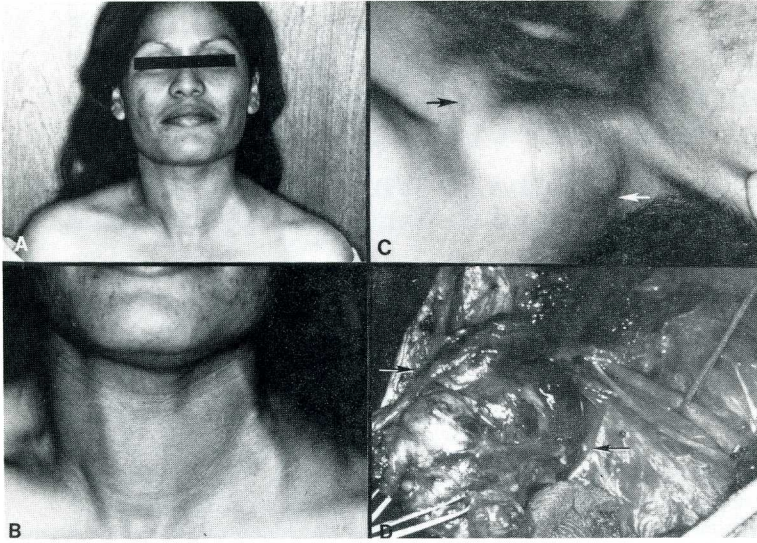
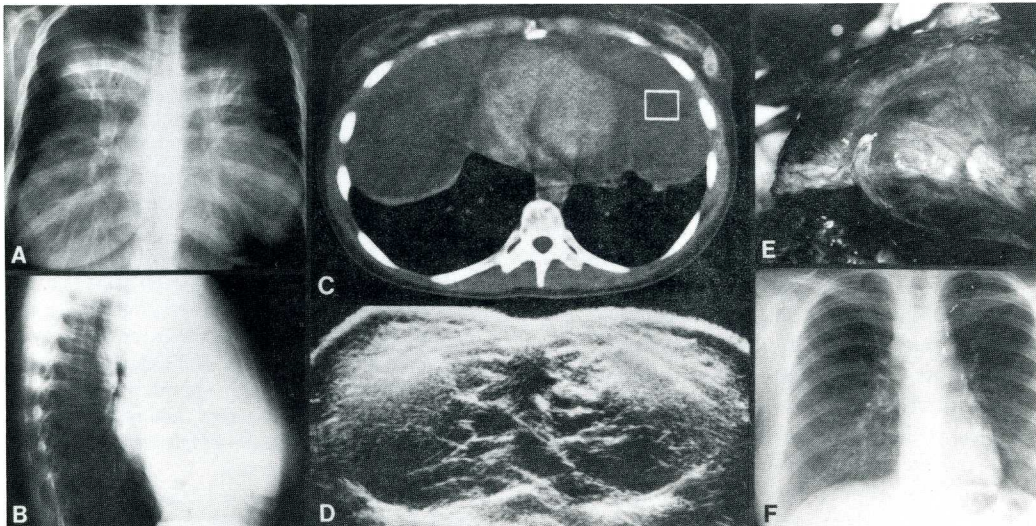


Fig. 1: Left supraclavicular fullness (A) with enlargement to a fluctuant mass with left external jugular venous distension after Valsalva maneuver (B). In the head-dependent position (C), the cervical mass is even more prominent and corresponds to intraoperative photograph (D). The lesion was dissected from the adjacent carotid sheath (loop tie) and phrenic nerve.

Fig. 2: Chest radiograph (A,B) demonstrating a huge space-occupying lesion in the anterior mediastinum with left neck and supraclavicular fullness displacing the trachea to the right. Computerized tomography (C) shows extent of thoracic encroachment by a uniform fluid-filled mass. Ultrasonography (D) discloses areas of dense echogenicity anteriorly consistent with multiloculated "cyst." Through a median sternotomy the mass appears dark red and cystic (E). Although numerous fibrous attachments to the chest wall and pericardium tethered the mass, there was no invasion or adherence to the heart or lungs. F. Follow-up chest x-ray 6 months later shows no evidence of recurrence of the hygroma.



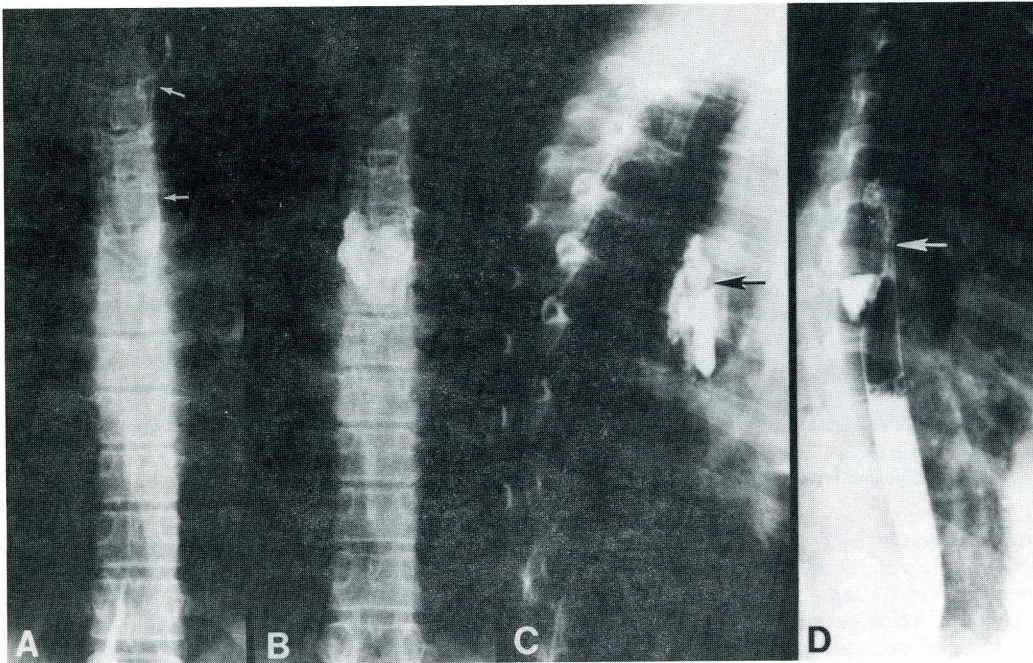


Fig. 3: Pedal lymphangiogram demonstrating normal thoracic duct (A) with a contained collection of contrast material (lymphocele) separate from the anterior mediastinal mass (B,C). Barium esophagram (D) shows the cyst with retained contrast compressing the esophagus at the level of the carina.

unremarkable. Quantitative serum immunoglobulins showed only mild elevation of immunoglobulin G. Assessment of pulmonary function disclosed a vital capacity of 1.7 liters indicative of moderately severe restrictive ventilation.

Computerized tomography with vascular enhancement (Fig. 2C) demonstrated an extensive cervicomedial mass extending superiorly into the left neck to the angle of the mandible including anterior and posterior cervical triangles and inferiorly to the diaphragm. The major portion extended to both lung apices encasing the great vessels and containing loculated fluid and punctate areas of calcification. Smooth regular margins favored a non-malignant process. Ultrasonography (Fig. 2D) revealed multiple sonolucencies within the mass separated by numerous septae.

Pedal lymphangiogram (Fig. 3A-C) disclosed cystic outpouching of an otherwise normal thoracic duct in the mid-posterior mediastinum, separate from the main mass anteriorly. Barium swallow (Fig. 3D) demonstrated upper esophageal compression from the ethiodol-filled cyst seen on the earlier lymphangiogram. Skeletal survey (Fig. 4A,B) revealed characteristic "soap bubble" lesions of lymphangiomatosis in the pelvis, iliac crests, proximal

femurs, anterior left 9th rib, 5th lumbar vertebrae and skull. These honey-combed defects failed to take up radionuclide (technetium 99m pyrophosphate) on bone scintiscan (Fig. 4C).

One-stage surgical resection was carried out through a left neck — sternal splitting incision. The tri-lobed multi-loculated mass (Fig. 2E) based at the left jugulo-subclavian junction was adherent to the pericardium and left lung hilus and encased the left phrenic and vagus nerves. One portion extended superiorly into the left neck (Fig. 1D) surrounding the carotid artery and internal jugular vein and was densely adherent to the cervical fascia overlying the brachial plexus. Although the bulk of the mass contained primarily serosanguinous fluid, a small cyst at its pedicle near the thoracic inlet was chylous. To minimize recurrent dysphagia, smaller cysts in the intrathoracic tracheoesophageal groove were also resected through a separate right thoracotomy.

The postoperative course was benign except for vocal squeakiness due to a compensated left vocal cord paralysis. Six months later she was asymptomatic, with a normal voice, clear chest radiograph (Fig. 2F) and stable bone lesions.

Blood-tinged fluid from the main large hygroma had a total protein content of 5.2 g/dl and an

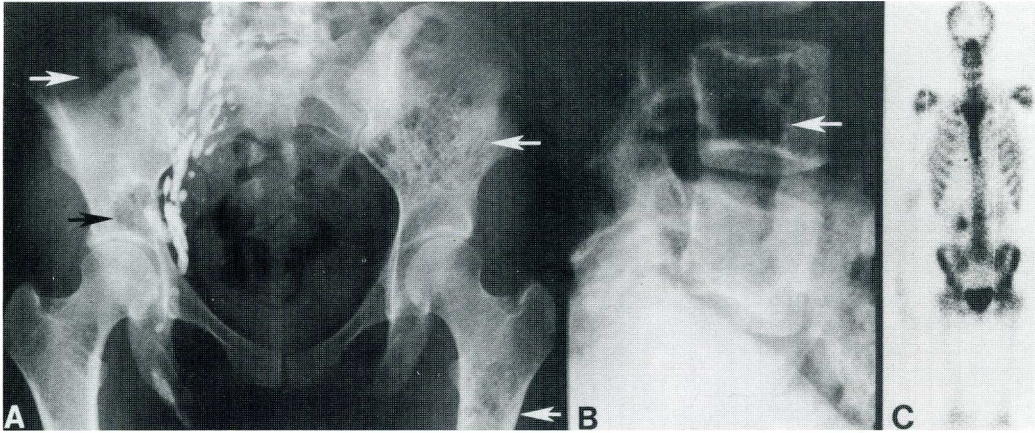


Fig. 4: Lytic "soap bubble" lesions typical of lymphangiomatosis involving both ilia, proximal femurs (arrows) (A) and lumbar vertebra (arrow) (B), which fail to visualize on radionuclide bone scintiscan (C).

albumin content of 3.4 g/dl while the chylous cyst exhibited values of 3.9 and 2.5 g/dl compared to serum values of 7.5 and 4.5 g/dl respectively. Triglyceride level in the main hygroma fluid was only 17 mg/dl but in the chylous cyst was 655 compared to a serum level of 67 mg/dl. Immunoglobulin G, A, and M levels were higher in the main hygroma (971,94,42 mg/dl respectively) than in the chylous cyst (490, 47, 1 mg/dl) but lower than levels found in serum (1530, 142, 105 mg/dl).

Histologic examination of the resected specimen (Fig. 5A) revealed a complex structure of irregular endothelium-lined cystic spaces intertwined with fibroadipose tissue and smooth muscle containing aggregates of lymphocytes, a picture identical to the lesion resected from her right neck 15 years earlier. The cyst lining gave a strongly positive immunoperoxidase reaction for Factor VIII-associated antigen (Fig. 5B), similar in intensity to the endothelium lining stromal blood capillaries. Transmission electron microscopy (Fig. 5C) revealed encircling the cyst an almost continuous layer of endothelial cells, which exhibited a thin discontinuous basement lamina and tight junctions but lacked Weibel-Palade bodies and pinocytotic vesicles; scattered below the endothelium were pericytes surrounded by a dense glycocalyx with abundant fine filaments and dense plaques. Radioimmunoassay for estrogen and progesterone receptors (1) in fresh tissue from the resected specimen revealed a progesterone receptor level of 45.8 f moles/mg cytosolic protein but no detectable estrogen receptors.

A representative portion of the resected hygroma was washed in sterile saline, cut into 1 mm³ "explants" using a sterile #10 scalpel, and placed

directly into Ham's F12 and Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum, 10% neonatal serum, HEPES, insulin, penicillin, streptomycin, and fungizone, seeded into Falcon T25 flasks, and cultured as previously described (2). After the initial outgrowth some explants were removed from the flask, trypsinized in .1% trypsin, .05% EDTA in PBS-A at 37°C and then reseeded and routinely maintained in T25 flasks. Each culture flask was examined under an inverted light microscope, and selective trypsinizations were carried out to remove non-endothelial appearing cell types as necessary. During the ensuing 18 months, the individual cell cultures have been successfully subpassaged from 3-6 times, maintaining a primary mixed cell line approximately 10-20% of which consists of polygonal endothelial-like cells seen at times to produce tubular structures typical of endothelium (Fig. 6A,C) and form "bean-shaped" nodules (Fig. 6A). These polygonal cells were decorated by both anti-Factor VIII-antibodies (Fig. 6B) and *Ulex europaeus* lectin (UEA-1) (3) (Figs. 6C,D), staining features distinguishing vascular endothelium from other cell types (3-5) such as fibroblasts found in the same culture.

Discussion

Lymphangiomas are arbitrarily categorized according to size of individual cysts and anatomic site of origin: 1. lymphangioma simplex or capillary lymphangioma of tiny thin-walled cysts 2. cavernous lymphangioma consisting of

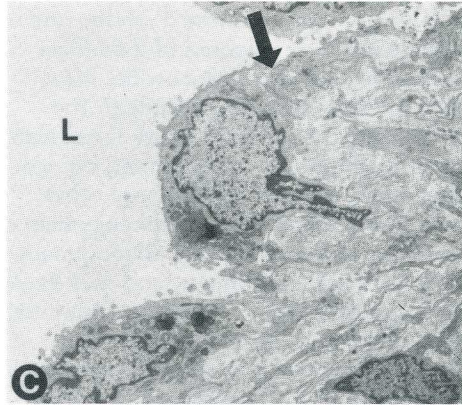
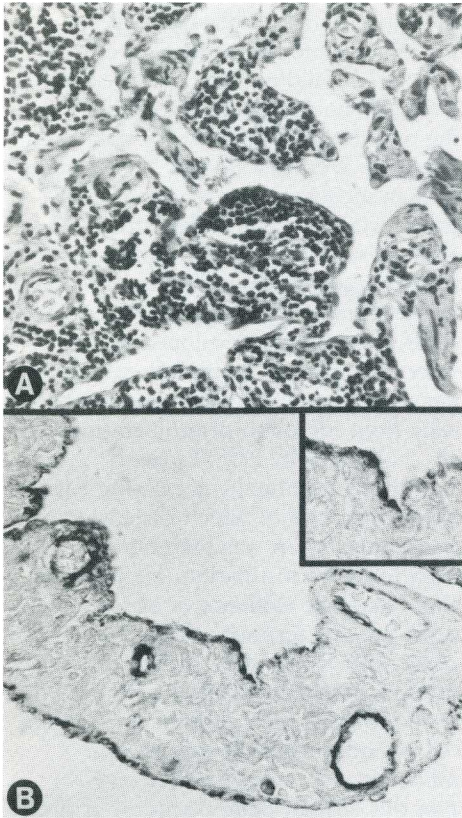


Fig. 5: Light micrograph (A) of the resected hygroma demonstrating complex endothelium-lined cysts with intervening aggregates of lymphocytes (H&E 160X). The cyst lining gives a positive immunoperoxidase reaction for Factor VIII-associated antigen (B) (PAP 250X, enlarged inset PAP 625X), similar in intensity to endothelium of blood capillaries in the vascularized stroma. Transmission electron micrograph (C) of characteristic endothelial cell lining the cyst. Note the fine discontinuous basement lamina and tight junction (arrow) visible even at the 3000X magnification.

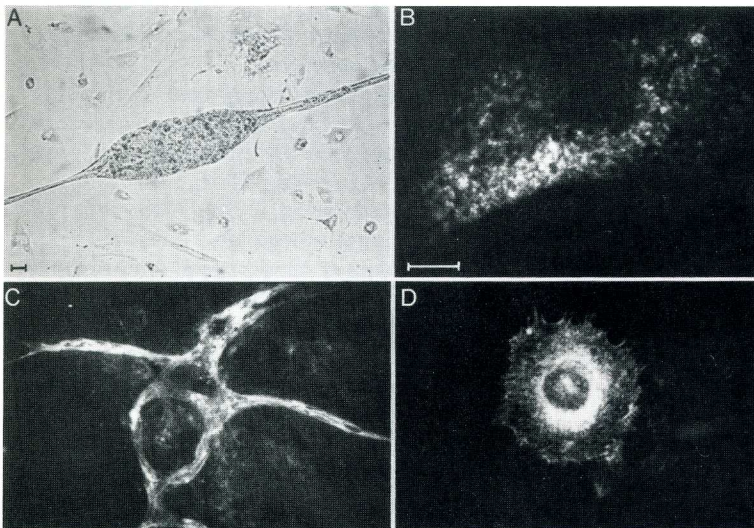


Fig. 6: Primary culture of the cystic hygroma shows a mixture of cell types (A) including bipolar spindle cells resembling fibroblasts and more polygonal cells, which form bean-shaped nodules (A) and are decorated with anti-Factor VIII antibody (B). Tubular structures (A,C) are observed throughout the culture medium and are brightly decorated with *Ulex europaeus* lectin (UEA-1) (C), which also decorates the surface and perinuclear region of the polygonal cells (D). Bar equals 10 microns.

larger, more dilated channels with denser fibrous lymphocytic infiltrated stroma and 3. cystic hygroma composed of fluid-filled sacs often several centimeters wide. Most cystic hygromas arise or protrude in the neck or axilla, and a small fraction are scattered throughout the mediastinum, cervico-mediastinum, retroperitoneum and other remote sites. Diffuse bony lymphangiomas are rare but characteristically asymptomatic, require no specific treatment, and may even resolve spontaneously. Sometimes, however, osseous dissolution develops with pathologic fractures, and when progressive and extensive, this phenomenon is termed "vanishing bone disease" (6,7).

Optimal treatment of the larger more complex cystic hygroma requires thorough anatomical delineation of adherence and infiltration to adjacent vital structures. In the past, these growths have been treated expectantly or by serial aspiration, injection with sclerosing agents, irradiation or multi-staged operations. As reported in a recent series (8) and confirmed in the present report, one-stage resection is technically feasible and indeed desirable even for giant strategically placed hygromas.

Apart from the dramatic clinical presentation and treatment, our patient raises fundamental questions about the origin of cystic hygromas and how they enlarge. While this lesion is commonly viewed as a "blind pouch" or "sequestered" lymphatic receptacle, i.e. strictly a developmental anomaly, the multifocal compartments containing distinctly different fluids suggest a varied embryologic origin and development. The chylous nature of the cyst at the left thoracic inlet, partial communication with the thoracic duct in the posterior mediastinum seen on lymphangiography, and the variety of fluids consistent with "central lymph" from different sites contained within the multicompartment mass, illustrate that cystic hygromas are not necessarily discrete and need not be entirely isolated from major lymph trunks.

Although blood-tinged fluid in this cervicomediastinal hygroma suggests some ex-

pansion due to intracystic bleeding, the massive enlargement after age 15 years, during which time the patient gave birth to three healthy children, and the prominent bony lesions point to true proliferation during adulthood. This concept was first proposed by Wernher (9) and later Virchow more than a century ago who regarded hygroma as a neoplasm rather than developmental anomaly (10). After endothelium was subsequently recognized as the predominant cell type lining the cyst, however, controversy developed as to whether enlargement derived from widening of preexisting lymphatic channels or alternatively from ingrowth of proliferating lymphatic capillaries. In 1938, Goetsch painstakingly examined the growing edge of 17 cystic hygromas in which distension of the cavernous lesion was maintained through formalin instillation. He adduced striking histologic evidence of active endothelial growth sprouting from the hygroma with penetration and destruction of adjacent tissues including formation of lymphoid follicles with germinating centers as well as endothelium-lined cysts surrounding smooth muscles and nerves. Despite this elegant demonstration of the independent power of irregular growth of these sequestered lymphatic rests, the concept of hygroma as a true neoplasm propagating from overgrowth of newly formed lymphatic endothelium has not been widely considered.

Human endothelium from umbilical vein and other large blood vessels was first successfully grown in tissue culture approximately 10 years ago and in 1979 from human capillaries (3,11,12). Although morphologic, immunologic and metabolic characteristics of blood vessel endothelium have now been more fully delineated, no previous publication deals specifically with *in vitro* growth of normal or pathologic lymphatic endothelium. The explant tissue culture technique used here combined with selective trypsinization as well as physical removal of unwanted cell types (2) has sustained for the past 18 months a mixed cell culture with a prime component of

polygonal cells bearing morphologic and biochemical characteristics of vascular endothelium. This endothelial subpopulation, representing nearly 20 percent of the total cultured cell population of roughly 20 to 30 million cells, forms networks of tubes with bean-shaped nodules and is brightly decorated with Ulex lectin (an antigen shared with Type H blood group) and anti-Factor VIII-antibody. Both markers are highly specific for vascular endothelium (3,4,11), and neither is found in smooth muscle cells or fibroblasts.

The identity of the endothelial cells in tissue culture and those in histologic sections of the hygroma is supported not only by morphologic similarity but also by the strong positivity for Factor VIII-associated antigen from both sources. Although disagreement exists about the presence of Factor VIII-associated antigen in lymphatic compared to blood capillary endothelium (5), this patient's hygroma had little or no hemangiomas component and displayed few red blood cells in cyst spaces, features favoring a pure lymphatic endothelial origin. Moreover, a single case report (13) of Factor VIII positive endothelium has been described in a lymphangiosarcoma arising in chronic postmastectomy lymphedema, and our unpublished observations indicate that endothelium from major lymphatic trunks of rats and dogs stain strongly positive for Factor VIII antigen.

The unexpected long-term survival of this endothelial cell line derived from the hygroma (human and bovine endothelium seldom remains in culture for more than a few months before senescence) (14) is of particular interest regarding the relation of the primary mass to diffuse osseous lesions, a rare but well recognized syndrome of generalized lymphangiomatosis. Are these bone lesions simply sequestered embryonic lymphatic channels representing blind sacs or are they embryonic "rests" responsive to the same stimulating growth factors as the main mass or possibly even distant implants as in endometriosis or trophoblast embolism? The hormonal influence of progesterone and estrogen in other blood

vascular and lymphatic disorders and the positive progesterone receptors in this patient's hygroma raise intriguing considerations in this regard. Hemangioma, a conglomeration of blood vascular channels closely related to lymphangioma, and occasionally occurring as a mixed lesion, waxes and wanes with pregnancy and menarche (5); pulmonary lymphangiomyomatosis, a lung disorder with histologic appearance similar to cystic hygroma, is exclusively found in fertile females and involved lymphatic endothelium may exhibit progesterone-positive receptors (15); and primary lymphedema with lymphatic hyperplasia may initially appear or worsen at puberty (lymphedema praecox). In addition to these observations suggesting hormonal influences in "benign" endothelial proliferation, long-standing primary or secondary lymphedema may undergo overt malignant endothelial transformation and proliferation. Moreover, massive or multiple lymphangiomas as well as hemangiomas may respond to the endothelium-paralyzing effects of ionizing irradiation (16), and after resection of a primary lesion, may spontaneously regress in distant sites such as bone (17,18).

Finally, there are also genetic overtones to cystic hygroma as suggested by familial syndromes and chromosomal abnormalities associated with such lymphatic abnormalities. Particularly notable in this regard is the high incidence of associated local and widespread disseminated lymphatic abnormalities including cystic hygroma in ovarian dysgenesis, Turner's syndrome (19). Eight percent of aborted fetuses with massive strangulating cystic hygromas exhibit Turner's syndrome, and indeed, the characteristic web neck is thought to derive from an involuted in utero cystic hygroma.

Thus, both genetic and hormonal influences appear to interact in the growth and development of the lymphatic system as well as in the appearance of lymphatic anomalies and tumors. Further, the distinction between sequestered embryonic rests and neoplasia is blurred as both can be associated with enlargement, multifocal le-

sions, encroachment, destruction of adjacent tissue and even death. The ability to pass this patient's cystic hygroma into tissue culture now permits in vitro examination of the morphologic development of human lymphatic vascular endothelium, its biochemical characteristics, and response to growth factors as well as alterations in these properties during proliferation. Clearly, the conventional view that these complex lesions are merely sequestered lymphatic receptacles unrelated to neoplasia seems an oversimplification.

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