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LYMPHANGIOGENESIS AND LYMPHOLOGIC SYNDROMES

MH Witte and CL Witte

Department of Surgery, University of Arizona College of Medicine, Tucson, Arizona

ABSTRACT

A unifying concept linking disorders of lymphatic dysplasia, hyperplasia and neoplasia is presented. The central role of disturbed lymphangiogenesis is illustrated in a wide variety of lymphologic syndromes characterized by lymphedema, lymphangiectasia, lymphangioma, and lymphangiosarcoma.

Lymphologic disorders characterized by edema, ectasia, tumor formation, and nodal dysfunction are usually classified separately. Yet commonly, two or more features are observed simultaneously (i.e., synchronously) or sequentially (i.e., metachronously), and occasionally all occur together. Based on the spectrum of congenital and acquired syndromes involving different components of the lymphatic system, we propose that lymphatic endothelial proliferation, that is, lymphatic angiogenesis or lymphangiogenesis, is a key process linking these diverse phenomena and governing both the clinical manifestations and biologic behavior. Whether in the aborted fetus with strangulating cystic hygroma associated with Turner's syndrome (gonadal dysgenesis), or the woman in her child-bearing years with progressively fatal pulmonary lymphangiomyomatosis, or the homosexual male with nodal angiofollicular hyperplasia then Kaposi's sarcoma (i.e., full-blown acquired immunodeficiency syndrome or AIDS), a fundamental biologic process—growth and development of lymphvascular endothelium—has become seriously disturbed.

To highlight and further embellish these concepts and their possible genetic, hormonal, and pathophysiological basis, we describe a group of patients with two or more of the following features: peripheral lymphedema, chylous or nonchylous effusions, visceral lymphangiectasia, pulmonary lymphangiomyomatosis, cystic hygroma or cavernous lymphangioma with and without diffuse bony lymphangiomatosis, and acquired immunodeficiency syndrome (i.e., AIDS) with disseminated Kaposi's sarcoma.

Genetic overtones emanate most vividly from the odd association of strangulating cystic hygromas (1) with Turner's XO-ovarian dysgenesis as in the hydropic, aborted, 16-week-old fetus shown in Fig. 1. Those surviving fetal life typically

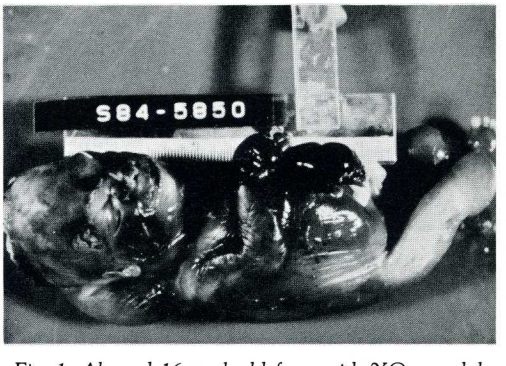

Fig. 1: Aborted 16-week-old fetus with XO gonadal dysgenesis (Turner's syndrome) and massive strangulating cervical cystic hygroma and generalized hydrops (whole body lymphedema).

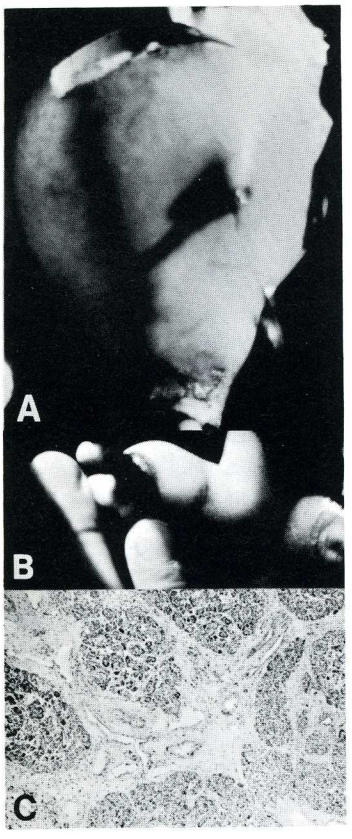

Fig. 2: Findings in a premature infant who died 5 days after birth with non-chylous ascites (A), genital and lower extremity high-protein edema and congenital lymphangiectasia. Instillation of blue dye into the first web space adjacent to the great toe remained as a solitary blob and failed to show "streamer formation" or forward flow (B). At autopsy, 2 days later, no blue dye was visible in retroperitoneal lymphatic trunks or an atretic thoracic duct. Histologic sections of the liver, kidney, and small bowel revealed numerous dilated irregular lymphatic spaces, and that of the leg disclosed edema but without discrete lymphatics. Microscopy of the pancreas (C) also revealed extensive lymphangiectasia and numerous fibrotic bundles interspersed throughout the parenchyma (x125).

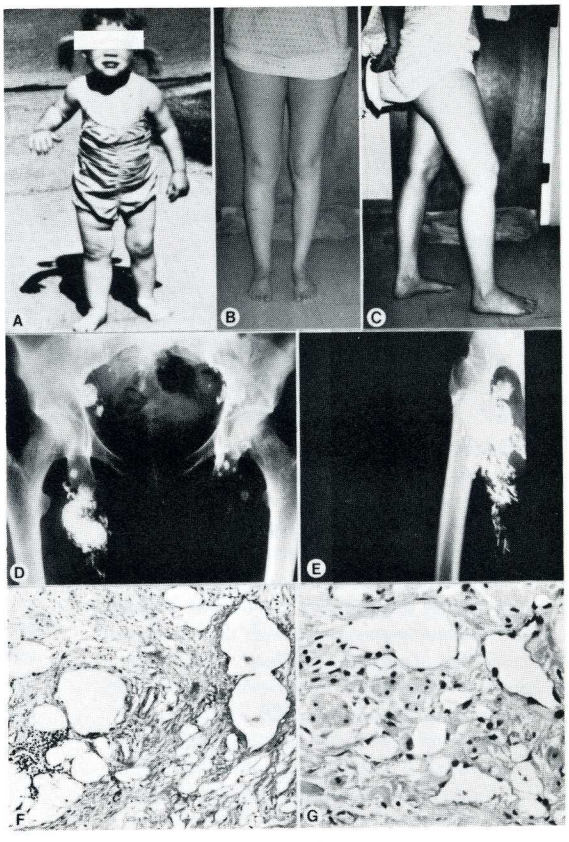

Fig. 3: Thirty-two-year-old woman with congenital, progressive lymphedema of the right leg associated with a large angiolipoma. Lower leg swelling was already apparent at 6 years (A) with a typical "milk-leg" as an adult (B,C). Pedal lymphangiography showed a large, subinguinal thigh mass supplied by normal lymph trunks (D) from which ethiodol extravasated (E). Histopathology was consistent with a proliferating lymphangiolipoma (F,G) (low, high power respectively).

demonstrate the characteristic webbed neck (pterygium colli) from in utero hygroma resolution as well as a high incidence of peripheral lymphedema. Among the congenital lymphedema syndromes, associated visceral lymphangiectasia (Fig. 2) is characteristically accompanied by effusion (both chylous and nonchylous) as in this neonate with Down's syndrome, who expired a few days after birth with multiple cardiac and intestinal anomalies, nonchylous ascites, peripheral lymphedema, and intraabdominal lymphangiectasia shown in a scarred pancreas. Klippel-Trenaunay and Maffucci syndromes are other congenital disorders of dysplastic endothelial development (both lymphangiomas and hemangiomas), which on rare occasion "degenerate" into angiosarcomas.

Angiomas, angiolipomas, angiofibromas,

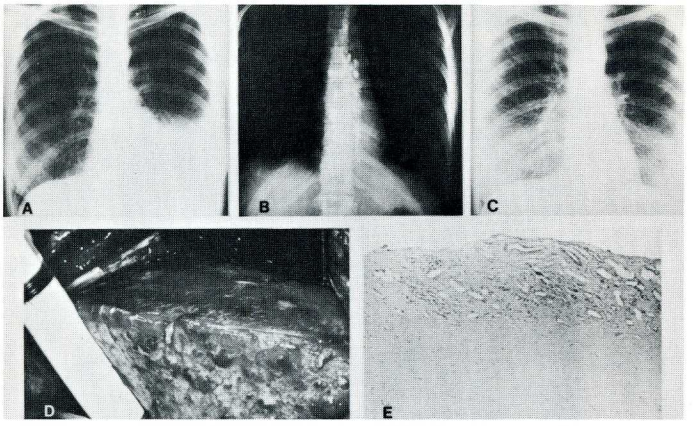

Fig. 4: Findings in a 27-year-old woman with pulmonary lymphangiomyomatosis. Initial presentation was shortness of breath and chest x-ray disclosed a large left pleural effusion (A). Peripheral lymphangiography revealed attenuation of retroperitoneal lymph trunks with mesenteric backflow and replacement of a discrete thoracic duct by multiple, irregular, small, mediastinal lymphatic channels (B). At time of pleurectomy (for pleurodesis), the lung showed extensive honeycombing (bullous emphysema), and the effusion was milky (white test tube) (D). Histology of resected pleura revealed marked lymphangiectasia (E, x45). Chest x-ray 5 years later, just prior to death from respiratory failure, showed progressive widespread fibrosis (C). (Reprinted from Edema, Raven Press, 1984) (3).

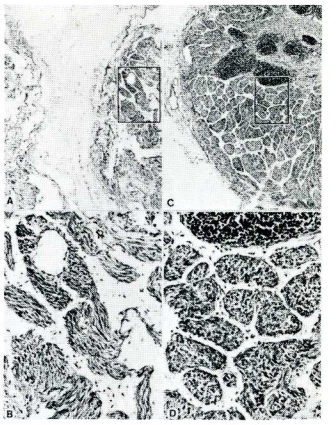

Fig. 5: Photomicrographs of lung (A,B) and hilar lymph node (C,D) in patient with lymphoangiomyomatosis (See Fig. 4) demonstrate extensive smooth muscle proliferation and lymphangiectasia. Lower micrographs (B,D) are higher power views of areas "boxed off" in upper sections (A,C).

and angiomyomas are variant tumors commonly observed in patients with congenital lymphedema as in the young woman depicted in Fig. 3 with right leg lymphedema since early childhood. During the past few years, peripheral edema had progressed proximally and pedal lymphangiogram displayed a "leaking" angiolipomyoma of the groin and extravasation of ethiodol into the thigh. Histologic examination revealed endothelium-lined lymphatic channels (which were positive as the adjacent blood vessels for endothelial marker Factor VIII-related antigen), smooth muscle bundles, and clusters of fat cells in the resected tumor. Lymph nodes and lymphatic channels above the tumor were reduced in number. Of related interest, patients with tuberous sclerosis, an autosomal dominant genetic disturbance associated with adenoma sebaceum and epileptic seizures, often develop multiple angiolipomas, particularly renal, with a subgroup of young women also exhibiting lung abnormalities indistinguishable from pulmonary lymphangiomyomatosis (2).

Endocrine overtones of these lymphologic syndromes derive from several considerations including the delayed appearance of primary lymphedema until puberty (so-called lymphedema precox) and the near exclusive incidence of pulmonary lymphangiomyomatosis in women of childbearing years. In the latter disorder, as exemplified in the patient in Fig. 4, pulmonary fibrosis and progressive anoxemia ended in death 5 years later. Initial presentation was with dyspnea and intractable ipsilateral chylothorax and later contralateral spontaneous pneumothorax necessitating bilateral pleurectomy. Lymphangiography disclosed an absent thoracic duct with mesenteric backflow while histopathology showed pleural lymphangiectasia. Post-mortem examination (Fig. 5) confirmed lymphangiomyomatosis with prominent smooth muscle proliferation both in the lungs and replacing adjacent hilar lymph nodes. Not only have progesterone receptors been identified on endothelial cells comprising these proliferating lesions (4), but oophorectomy, progestational agents, and antiestrogenic compounds such as tamoxifen have been advocated to retard

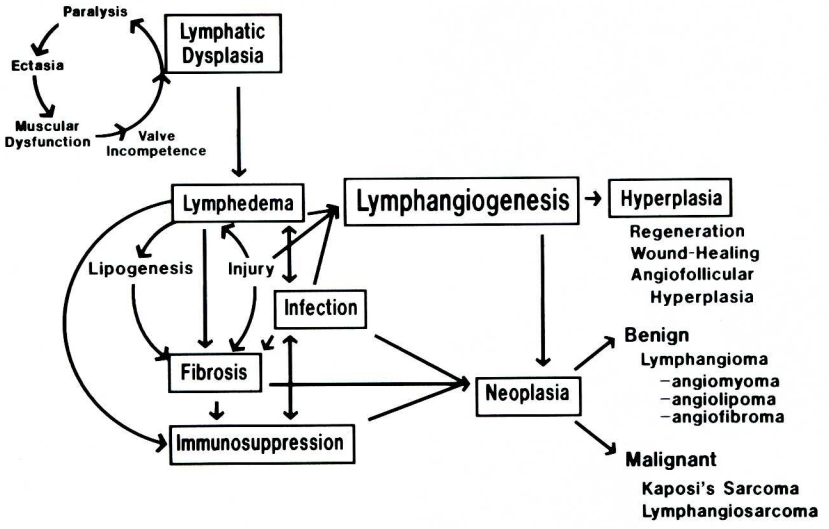

Fig. 6: Schematic diagram proposing pathophysiologic links between lymphangiogenesis and lymphangiodysplasia, hyperplasia and neoplasia. During a latent period, lymphatic dysplasia produces ectasia, muscular dysfunction, paralysis, and valve incompetence culminating in persistent high-protein lymphedema. Subsequently, superimposed opportunistic infection, immunosuppression, and injury with recurrent lymphangitis promote persistent lymphedema, intense angiogenesis, lipid deposition and fibrosis, and on rare occasions malignant vascular neoplasia. This sequence of events may occur as a local phenomenon in peripheral lymphedema or in a multitude of isolated or generalized visceral disorders characterized by scar formation and angiohyperplasia and neoplasia. See text for further details.

the inexorable downhill course of this disorder. In this context, it is noteworthy that estrogens stimulate endothelial cell growth in vitro while oral contraceptives and other estrogen compounds are implicated in a variety of vascular abnormalities such as ectasias, peliosis, and angiomas (5). Moreover, oral contraceptive drugs and hyperestrogenemia of pregnancy both appear to promote growth of lymphangiomas or worsen the clinical manifestations of visceral lymphangiectasia.

Whether the various components of these lymphangiopathies are rooted in genetic defects aggravated by hormonal imbalances or alternatively arise indirectly through the final common pathway of longstanding lymphostasis is unclear. Nonetheless as an outgrowth of congenital lymphatic hypoplasia or after cancerous invasion or surgical excision of regional lymph nodes, a well-recognized sequence of events follows inadequate lymphatic drainage and includes proliferation of lymphatic endothelium and progressive lymphangiectasia during a lengthy latent period before the onset of persistent lymphedema (Fig. 6). Lymphatic truncal walls gradually thicken in a dense network of irregular channels forming vesicles as well as deep-lying varicose lymphatics assuming angiomatoid configurations (7,8). As high-protein lymphedema persists and deposits of fat accumulate, progressive interstitial fibrosis leads to brawny induration. Repeated cellulitis and lymphangitis ("opportunistic infection") commonly supervene with ongoing breakdown of local tissue immunity. In the extreme, grotesque elephantiasis with hypertrophic skin bosellations appears, and on occasion fatal lymphangiosarcoma develops in the affected limb.

Clinically and histopathologically, there exists a fine line between congenital malformations and true neoplasia and between benign and malignant growth. In cystic hygroma, the degree of vascular proliferation, extracellular matrix deposition, lymphoid aggregation, and "tumor" invasion of nearby structures raises fundamental ques-

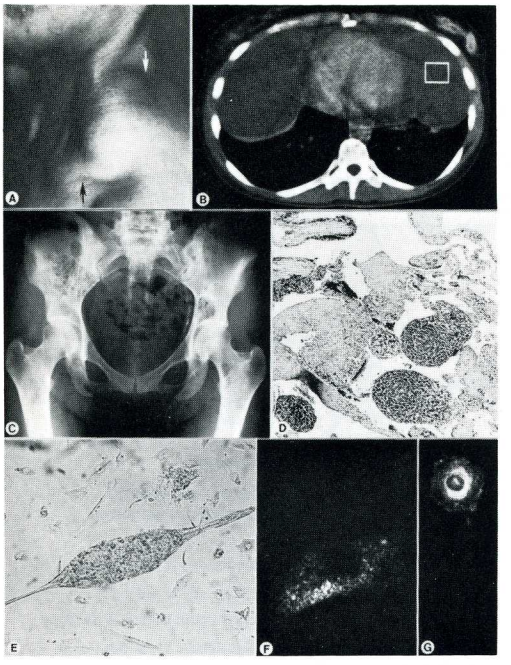

Fig. 7: Twenty-nine-year-old woman with left supraclavicular fullness (A) from massive recurrent cervico-mediastinal cystic hygroma (computerized tomography, B) with lytic "soap bubble" lesions of bony lymphangiomatosis (C). Resected tumor histology (D) showed complex endothelium-lined cysts and intervening lymphoid aggregates. Primary tumor culture sustained long-term a mixture of cell types including fibroblasts and endothelial cells, the latter forming tubular structures (E) strongly positive for endothelial marker Factor VIII-associated antigen (F) and polygonal cells brightly decorated with Ulex europeus lectin (UEA-1) (G) (Modified from Lymphology 17 (1984), 15) (9)

tions about the pathophysiology of such multiloculated "growths". These concepts are illustrated in the following female patients—the first, a 29-year-old woman with relatively sudden and massive enlargement of a cervicomediastinal cystic hygroma associated with widespread skeletal lymphangiomata from whom a mixed cell line including endothelium has been cultivated in tissue culture (9) (Fig. 7). The second is a 22-year-old woman with a rapidly expanding, giant, recurrent retroperitoneal chyle-filled cavernous lymphangioma that originated from the root of the small bowel mesentery adjacent to the uncinate process of the pancreas (10). The resected tumor showed a complex structure of regular cystic spaces intertwined with fibroadipose tissue, smooth muscle, and nodular aggregates. Both cyst lining and cultured endothelial cells from the tumor were positive for endothelial marker Factor VIII-related antigen and showed abundant fibronectin. A continuous monolayer culture of endothelial cells has been maintained for nearly 2 years without addition of growth factors. Transmission electron microscopy revealed relatively smooth cell surfaces with occasional microvilli, abundant Weibel-Palade bodies (presumed storage sites for Factor VIII-associated antigen), overlapping junctions, macula adherens and numerous bundles of intermediate filaments characteristic of lymphatic endothelium. The final patient is a 27-year-old woman (11) with a "benign" recurrent ovarian lymphangioma of the ovary which "seeded" the peritoneum with rapidly proliferating lymphvascular endothelium in a matrix of intense collagenosis and nodular aggregates of lymphocytes, features more compatible with vigorous "new" growth than congenital malformation with blind sacs of sequestered lymph.

The ability to establish in two of these young women primary lymphvascular endothelial cell lines in tissue culture without addition of exogenous growth factors typically required for culturing blood vascular endothelium, and the resemblance of endothelial cells in vitro to those lining the cysts of origin in vivo strongly favor independent endothelial growth potential in these lymphangiomas. Osseous lymphangiomatosis likewise may represent "new" growth rather than mere multicentric embryonic rests (9), particularly when regression occasionally occurs spontaneously (12). The relation of these phenomena to genetic defects or viral-transformed DNA, to control by female hormones, to the basic tissue response of lymphatic regeneration after injury, and to lymphatic obstruction, suggests common factors regulating proliferation of lymphatic endothelium or lymphangiogenesis. The boundary between benign and malignant

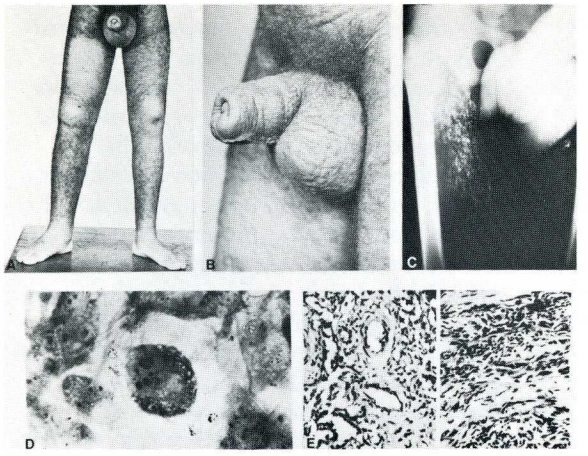

Fig. 8: Initial appearance (A,B) of a 15-year-old male demonstrating pronounced swelling of the penis, scrotum, and lower extremities. Genitalia exhibited brawny edema, and the penis and scrotum had minute cystic swellings representing lymphatic vesicles (B). Lower extremity lymphangiography (C) demonstrated obstruction of leg lymphatics at the groin with retrograde extravasation of contrast material (dermal backflow). Photomicrographs (D) of irradiated McCoy tissue culture of ascitic fluid obtained at postmortem, demonstrated "initial bodies" (dark cytoplasmic blobs), an intracellular phase of Chlamydia (x750). Photomicrographs (E) of groin subcutaneous tissue showed abnormal lymphatics lined by atypical endothelial cells with considerable pleomorphism and hyperchromatic nuclei (x416) consistent with "lymphangiosarcoma". (Modified from Lymphology 6 (1973), 113) (13)

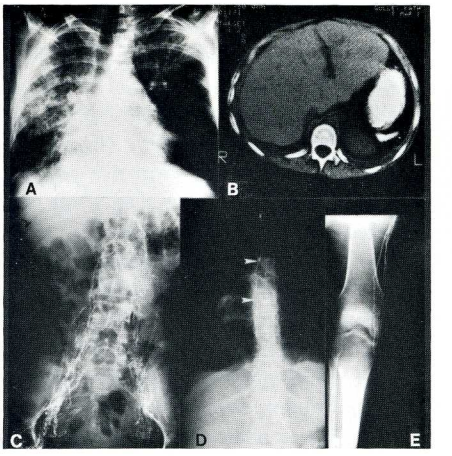

Fig. 9: Chest x-ray (A), abdominal computed tomography (B), pedal lymphangiography (C-E), in a 24-year-old woman with recurrent pulmonary infection and protein-losing enteropathy. Note progressive lung fibrosis (A), absence of the spleen (B), involution of paraaortic lymph nodes with "dysplastic" retroperitoneal lymphatics (C), a normal thoracic duct (arrowheads-D), and intact peripheral lymph trunks (E). (Modified from Lymphology .18 (1985) 31) (15)

vascular growth is further clouded by angiomatoid changes in both Kaposi's sarcoma and angiosarcoma arising in a lymphedematous limb, so-called Stewart-Treves syndrome. Perhaps related is the phenomenon of progressive, proliferative endothelial change in the liver ranging from huge vascular "lakes" (peliosis) to highly aggressive malignant angiosarcoma from overexposure to industrial monomers of vinyl chloride (5).

Finally, the entire spectrum of pathologic features involving all components of the lymphatic system—channels, fluid, nodes, and cells—appears together or sequentially in AIDS, the prototype acquired immunodeficiency syndrome. In 1968 we encountered a peculiar constellation of findings in a 14-year-old black man (13) beginning with scrotal and penile lymphedema and inguinal lymphadenopathy later followed by bilateral obstructive lower extremity lymphedema, which over a 2-year period progressed to whole body lymphedema, massive ascites, and bilateral pleural effusion (Fig. 8). Systemic chlamydial infection from a lymphopathia venereum-related organism ("opportunistic infection") was identified and despite broad spectrum antibiotics and diuretic drugs the patient sustained progressive inanition, immunosuppression pulmonary infection, and died. At post-mortem, not only was Kaposi's sarcoma (alternatively diagnosed as lymphangiosarcoma by different pathologists) found in a localized cutaneous lesion on the thigh, but it was also disseminated throughout the subcutaneous tissue and viscera. Particularly notable were lesions in the thymus and in lymph nodes, where reticuloendothelial hyperplasia and granulomas first observed more than a year earlier had given way to lymphocyte depletion, diffuse fibrosis, and angiosarcoma. This report lay dormant until 1984 (14) when we called attention to its close resemblance to AIDS:—namely aggressive Kaposi's sarcoma associated with opportunistic infection and

immunosuppression following a hyperplastic lymphadenopathy stage, so-called pre-AIDS. In other words, lymphedema, lymphangiectasia, lymphangioma, lymphangiosarcoma, and nodal dysfunction were all present in sequence in this patient, and not just localized to an extremity ("local AIDS") but generalized. Pieces of the picture occur in other patients with acquired and congenital immunodeficiency disorders such as in our recent report of a 23-year-old woman (15) with a long history of recurrent respiratory infections, pleural effusions, and hypogammaglobulinemia, who was noted at laparotomy as a teenager to have hyperplastic mesenteric lymph nodes but later developed chylous ascites, peripheral edema, vanishing spleen, and complete involution of abdominal lymph nodes (Fig. 9). Reviewing African Kaposi's sarcoma, a more indolent disease commonly heralded by peripheral lymphedema, Dorfman (16) has proposed that lymphatic endothelium gives rise to the malignancy. Findings of lymphedema and angiofollicular lymphadenopathy early in AIDS, angiolipomas, lymph node depletion and fibrosis late, and death from disseminated Kaposi's sarcoma, may reflect both the broad spectrum and biologic aggressiveness of endothelial proliferation.

In conclusion, these patients suggest that lymphatic endothelial proliferation (lymphangiogenesis) holds the key to biologic and clinical behavior of these lymphologic syndromes (Fig. 6). Where turnover of endothelium is slow and edema of limited magnitude and duration, benign processes such as cavernous lymphangioma, cystic hygroma, angiolipomyoma, and nodal angiofollicular hyperplasia are characteristic. While generally quiescent, these "tumors" may have multifocal sites of origin and given the appropriate genetic environment (e.g., an in-born error or viral DNA transformation) may enlarge and create local symptoms by distension or encroachment. In these or related lymphangiopathies such as lymphedema precox or pulmonary lymphangiomyomatosis, hormonal influences may foster development of ectasia, edema, effusion, and proliferation of various cell types related to endothelium. When carefully autoregulated as during repair after external injury, lymphangiogenesis reestablishes lymphatic continuity, and signs of insufficient tissue fluid drainage disappear. On the other hand, when unrestrained, as in Stewart-Treves syndrome, lymphangiogenesis culminates in highly lethal lymphangiosarcoma. Some of these phenomena are precipitated or aggravated by lymph nodal structural changes such as lymphocyte depletion with diffuse fibrosis. When locally confined, lymphedema and lymphangiectasia are usually limited in their effect, but when combined with systemic immunoincompetence (e.g., sexually transmitted AIDS virus), lymphangiogenesis escapes regulation and endothelial "regeneration" escalates into fatal Kaposi's sarcoma. Although these concepts are still speculative, it is reasonable to link the different components of the lymphatic system—lymphatics, lymph, lymph nodes, and lymphocytes—into a functional whole. With tools now available to examine structure, reactivity, and growth potential of lymphatic as well as blood vascular endothelium, both in vivo and in vitro, the process of lymphangiogenesis, the lymphangiogenic factors controlling it, and the mysterious interactions of endothelium with surrounding extracellular matrix and neighboring macrophages, smooth muscle fibroblasts, adipocytes, and lymphoid cell populations may now be addressed.

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