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Systemic Chlamydial Infection Associated with Generalized Lymphedema and Lymphangiosarcoma

M. Elvin-Lewis, Ph.D., Marlys Witte*, M.D., Charles Witte*, M.D., W. Cole, M.D., and J. Davis*, M.D.**

Department of Microbiology, Washington University School of Dentistry, and the Unit I (Washington University) Surgical Service, St. Louis City Hospital, St. Louis, Missouri

Summary

A Chlamydial agent was isolated repeatedly from a variety of body fluids in a Negro adolescent boy with a fatal progressive lymphedema and widespread lymphangiosarcoma. The hybrid Chlamydial organism cultured resembled closely *Chlamydia trachomatis*. The exact relationship of the infection to the lymphedema and lymphangiosarcoma in this patient remains to be clarified.

Introduction

Chlamydial organisms (previously classified as viruses and Bedsoniae) are recognized causative agents of cat scratch fever and lymphogranuloma venereum and have also been implicated in a whole host of obscure multi-system disorders (14, 20, 22). These organisms characteristically are in a constant state of genetic flux (16). Whereas lymphogranuloma venereum has been shown to be produced by either *C. trachomatis* (15) or *C. psittici* (18), the causative agents of cat scratch fever and a variety of Chlamydia-associated infections have yet to be classified by species.

Occasionally, cat scratch fever or lymphogranuloma venereum produces a fulminant regional lymphangitis and lymphadenitis which culminates in chronic lymphedema of the genitalia and lower extremities (5, 21). The following case report describes the clinical course of an adolescent boy who developed progressive generalized lymphedema starting in his genitalia and legs associated terminally with widespread lymphangiosarcoma. During this period, a Chlamydial organism sharing species characteristics of both *C. trachomatis* and *C. psittici* was repeatedly isolated from a variety of tissues and body fluids.

Present address:
*Drs. M. Witte, M.O. and Ch. Witte, M.D., Departments of Surgery and Pathology (Dr. Davis), University of Arizona College of Medicine, Tucson, Arizona 85724
**M. Cole, M.D., Department of Surgery, University of Missouri School of Medicine, Columbia, Missouri 65201

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Reprint requests to Dr. Memory Elvin-Lewis, Chairman, Department of Microbiology, School of Dentistry, Washington University, St. Louis, Missouri 63110

Case Report

A fifteen year old Negro boy was admitted to St. Louis City Hospital for penile and scrotal swelling and progressive edema of the legs (right > left) of 5 months duration. He denied injury or animal bites, travel outside the midwestern United States, or a family history of edema, although he admitted to frequent sexual intercourse. Physical examination revealed moderate mental retardation, prominent "woody" non-pitting brawny edema of the penis, scrotum, lower extremities, and abdominal wall (Fig. 1).

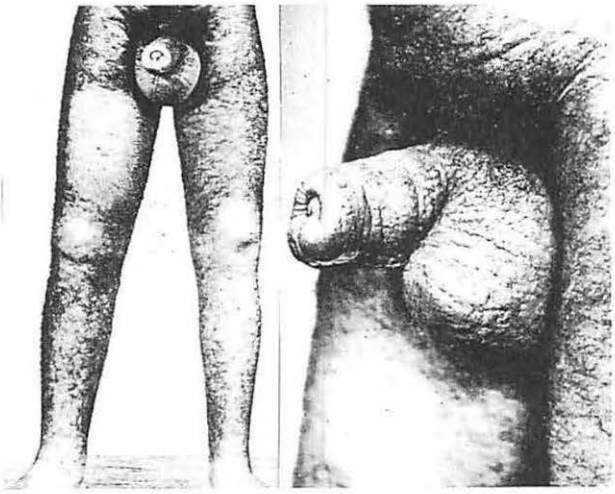

Fig. 1. Initial appearance of patient demonstrating pronounced swelling of penis, scrotum, and lower extremities (left). Genitalia (right) exhibited brawny "woody" edema and minute cystic swellings constituting dilated lymphatic spaces found on histologic examination

No lymph nodes were palpable. Laboratory findings indicated a normal hemogram and urinalysis. Right femoral vein puncture yielded a normal venous pressure and venography demonstrated a patent iliac vein and inferior vena cava. Lower extremity lymphangiography disclosed dilated peripheral lymphatic channels, dermal backflow, and complete obstruction to lymph flow at the inguinal ligaments in both legs (Fig. 2). After biopsy of an inguinal lymph node, histologic examination demonstrated marked fibrosis and a solitary non-caseating granuloma.

During the next few months the edema persisted despite dietary salt and water restriction, elevation and wrapping of the extremities, and administration of diuretic drugs. Further evaluation included a left supraclavicular lymph node biopsy which showed chronic granulomatous lymphadenitis on histologic examination. Thoracic duct cannulation in the left neck revealed an enlarged (1 cm diameter) thick-walled thoracic duct with a normal flow of 1.0 ml/min of chylous lymph. Thoracic duct lymph protein content was 3.5 gm% and clear edema fluid from the lower extremities contained 3.0 gm% of protein (Plasma protein 6.5 gm%). Microscopic examination of a skin biopsy from the edematous right leg revealed moderate fibrosis, marked edema of the corium, and marked lymphatic dilatation. Cytologic examination of thoracic duct lymph was negative for tumor cells. Although routine bacterial and fungal cultures of thoracic duct lymph were negative, Chlamydial organisms were isolated in heavy growth from thoracic duct lymph but not plasma. Later, Chlamydial organisms were also isolated from edema fluid, prostatic secretions, conjunctival secretions, plasma and other body fluids (see Special Studies). A Frei skin test was negative as was the inter-

Fig. 2. Lower extremity lymphangiogram (contrast material ethiodol) demonstrating bilateral obstruction of leg lymphatics at the groin with retrograde extravasation of dye (dermal back flow).

mediate tuberculin skin test. Serum complement fixation titers for filariasis were within normal limits and repeated smears of serum were negative for microfilariae. Over the next few months, lymphedema progressed upwards along the abdominal wall and a right pleural effusion developed. Thoracentesis yielded clear straw-colored fluid with a protein content of 3.5 gm%. A seven-week course of antibiotic drug therapy including tetracycline, penicillin, sulfasoxasole and cephalothin was instituted, but the patient continued to deteriorate. His terminal five months were marked by wasting of muscle mass, fibrosis of the skin and subcutaneous tissues and progressive subcutaneous lymphedema and serous effusions. Eventually almost his entire subcutaneous compartment was converted into a continuous space which exhibited a fluid wave. Needle puncture of the subcutaneous tissue at any point produced a steady stream of edema fluid which drained from the lower two-thirds of the body and both pleural spaces. Nearly two years after his initial hospital presentation, the patient died from inanition, intractable fluid imbalance and pulmonary insufficiency.

Autopsy revealed a wasted Negro boy with greatly thickened "elephantine" skin of the lower trunk, scrotum and penis. Microscopic examination disclosed widespread lymphatic dilatation and disseminated lymphangiosarcoma, particularly prominent in the lungs, peribronchial lymph nodes, scrotal tissue, thymus gland, sternothyroid muscle, diaphragm, bone marrow, thoracic duct, pleura, and rectum. Angiosarcomatous change of the skin was extensive and a skin nodule obtained from the left thigh showed a fibrosarcoma-like proliferation with invasion of dermal papillae and extensive acanthosis of the underlying epidermis with early bulla formation (designated as "Kaposi's sarcoma"). In typical sections of subcutaneous tissue (Fig. 3) and skeletal muscle from the groin (Fig. 4), myriad lymphatic channels with irregular lumina were seen in association with areas of fibrosis, diffuse lymphocytic and mononuclear cell infiltrate (Fig. 4 left). The more angiosarcomatous form of the neoplasm showed proliferating lymphatic cleft-like channels of very variable size and shape, which, as in inguinal subcutaneous tissue (Fig. 5), were lined by atypical endothelial cells exhibiting considerable pleomorphism and hyperchromatic nuclei. There was marked lymphocytic and mononuclear cell infiltrate and whorls of fibroblast-like cells. The reticulum, however, was less organized than in most lesions classified as Kaposi's sarcoma.

Fig. 3. Photomicrograph ob subcutaneous tissue of right lower extremity showing irregular pattern of lymphatic channels (H&E 65 X).

Special Studies

Microbiology – Speciments of body fluid and tissues were collected throughout the duration of the illness and at autopsy. Modified sucrose potassium glutamate (MSPG) was added as a diluent to preserve Chlamydial agents (23). To reduce bacterial contamination, an antibiotic mixture to equal 5 mgm of Neomycin and 0.25 mgm each of Kanamycin and Neomycin per ml incubated for four to eighteen hours at 4°C added to the samples. Tissue specimens were extracted by one cycle of freezing at -70°C, thawed at room temperature, and homogenized in Broeck tissue grinders. When not processed for inoculation immediately after collection, specimens were stored at -70°C. Six to eight day old fertile eggs were inoculated with 0.3 ml of this prepared specimen and incubated at 33.5°C. Eggs were candled daily and those dying after two days were harvested and tested for infectivity and sterility according to standard procedures. Living eggs were harvested on the twelfth to fourteenth day of incubation, yolk sacs homogenized, and blind passages carried out before a specimen was considered negative. A smear of each yolk sac, stained by either May Grunwald Giemsa (17) or Gimenez (7) methods was examined microscopically for the presence of initial or elementary bodies of *Chlamydia.* The agents were further identified by direct or indirect immunofluorescent techniques (13). Positive yolk sacs were stored at -70°C. Tissue culture propagation was carried out using irradiated McCoy tissue culture (8). After irradiation and infection, cells were grown in growth media (EMG) consisting of Eagle's minimal essential media with the addition of 30 μmoles of glucose per ml and an extra one per cent MEM vitamins, glutamine, non-essential amino acids and ten per cent fetal calf serum. To reduce bacterial contamination during isolation procedures, an antibiotic mixture was added to make a concentration of 50 μgm of Streptomycin and 25 μgm each of Neomycin and Kanamycin per ml of medium. The inoculum consisted of EMG containing ten to twenty per cent by volume of the test specimen which was added in one ml amounts to irradiated McCoy monolayers on cover slips in culture vials. After centrifugation incubation was carried out at 33.5°C for up to ninety-six hours. Cover slips were removed at intervals of twenty-four hours and stained with Lugol's Iodine and May Grunwald Giemsa and examined for glycogen-staining inclusions. Cultures incubated for 72 and 96 hours were sonicated for five minutes in a Sontegrator System Forty (Ultrasonic Industries, Inc.) and a portion was reinoculated into tissue cultures of the yolk sacs of fertile eggs.

Chlamydia were recovered as indicated in Table 1. The susceptibility of either yolk sac or irradiated McCoy tissue culture appeared to be the same. Isolation in first passage was considered to reflect a higher concentration of the agent than isolation on

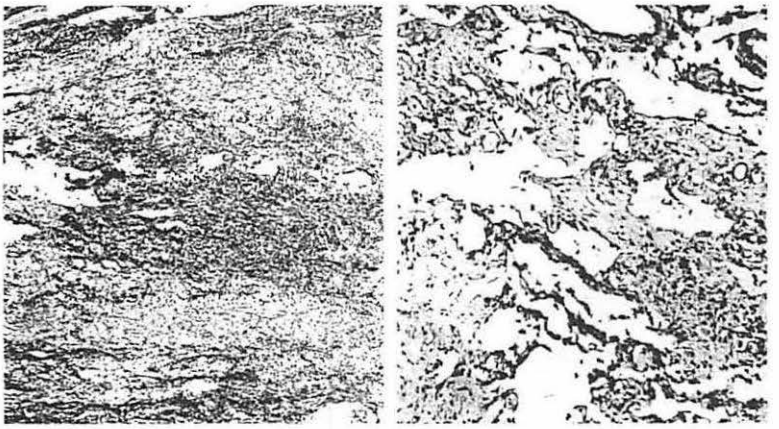

Fig. 4. Photomicrographs of skeletal muscle from groin showing fibrosis, diffuse lymphocytic and mononuclear cell infiltrate (left) and myriad irregular dilated lymphatic channels (right) (H&E 65 X and 166 X respectively).

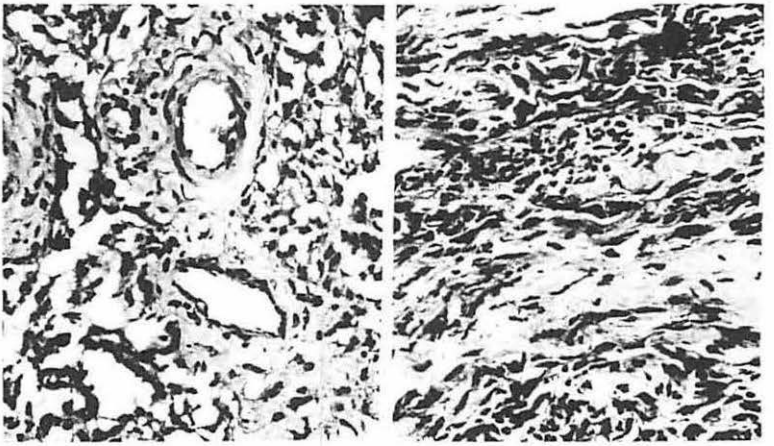

Fig. 5. Photomicrographs of inguinal subcutaneous tissue showing abnormal lymphatics lined by atypical endothelial cells (left) which exhibit considerable pleomorphism and hyperchromatic nuclei (right) (H&E 416 X).

second or third passage. Early in the patient's disease, agents were readily isolated from either lymph or edema fluids. As the disease progressed the Chlamydia increased in blood sera. At autopsy the organism was found widely disseminated throughout the body.

Microscopic morphology of the organism indicated well-defined rigid inclusions which stained with glycogen (Fig. 6). Initial bodies (Fig. 7) and elementary bodies (Fig. 8) were demonstrated in irradiated McCoy tissue culture material obtained at post-mortem examination.

Group-specific antigen was indicated by the isolate's ability to fix complement with

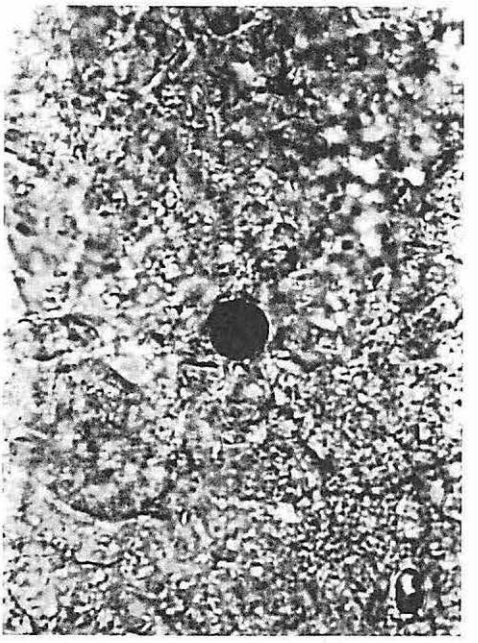

Fig. 6. Photomicrograph from irradiated McCoy tissue culture of serum obtained post-mortem examination demonstrating typical glycogen-staining inclusion characteristic of Chlamydial organisms (750 X).

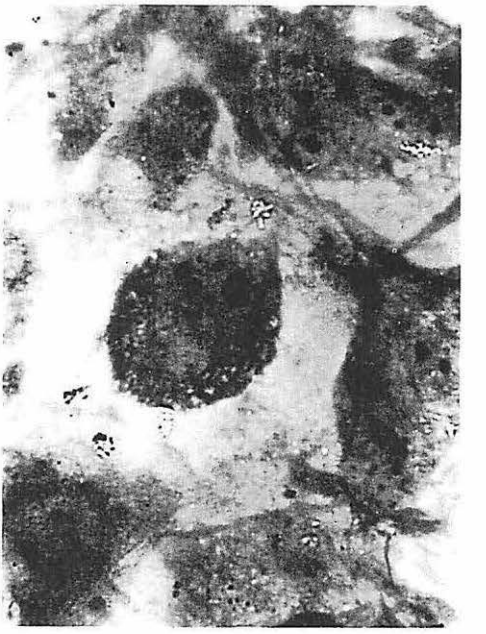

Fig. 7. Photomicrographs of irradiated McCoy tissue culture of ascitic fluid obtained at post-mortem examination demonstrating "initial bodies" (dark cytoplasmic blebs) representing intracellular phase of Chlamydial organism (750 X).

commercial (Markham) lymphogranuloma venereum antiserum and immunofluorescence. Sulfadiazine sensitivity was equivocal. Inoculations into animals using the fifth yolk sac passage agent originally isolated from edema fluid at an EID dose of 3.5 indicated low animal pathogenicity and no deaths occurred among ten mice inoculated intracerebrally, intravenously, or intranasally or a guinea pig inoculated intraperitoneally. Only two of ten mice died when inoculated intraperitoneally. The organism thus resembled Chlamydia trachomatis more than Chlamydia pscittici.

A variety of serologic tests which have been modified in Dr. M. *Elvin-Lewis'* laboratory were carried out on serum obtained during the patient's illness and at post-mortem examination. Using commercial lymphogranuloma venereum antigen (Markham Laboratory, Chicago) and a sensitive microtiter technique, group complement fixation titers were determined. As Chlamydia were isolated from all these sera it is likely that the low titers found, which never exceeded 1:8, represented only unbound antibody. Similarly, agglutinin titers, although not identical to complement-fixing titers, were also low (1:2) during the patient's illness and reached their highest titer at death (1:16), a time coincidental with the appearance of precipitin antibodies demonstrated by the Ouchterlony technique. As certain herpesviruses may be potentially oncogenic, his sera was also tested for the presence of antibodies to EBV virus (Burkitt's lymphoma) (11) and cytomegalovirus (Kaposis's sarcoma). No antibodies to EBV virus were detected and those to cytomegalovirus remained at low levels, never exceeding 1:4.

Fig. 8. Photomicrograph of irradiated McCoy tissue culture of brain at post-mortem examination demonstrating, partially obscured by overlying cell, elementary bodies (small dark dots) free in extracellular phase and in cytoplasmic inclusion (750 X).

Discussion

The clinical picture of venereal contact, initial involvement of the genitalia progressing to lymphedema of the lower extremities accompanied by lymph nodes containing granulomata is compatible with Chlamydial infection. The lack of a positive Frei skin test (performed late in the patient's course) may reflect extensive Chlamydial dissemination, lymphangiosarcomatous involvement of the thymus gland, or failure of cross-reactivity of antigens of this Chlamydial species with Frei antigen. The early phase of this patient's illness also resembles "non-venereal sclerosing lymphangitis of the penis", a peculiar entity thought to be caused by a viral agent (10). The fulminant course and generalized distribution of the edema is unusual for any form of lymphedema, congenital or acquired.

Whether Chlamydial infection played a primary or secondary role in the lymphedema and lymphangiosarcoma in this patient is conjectural. Chronic lymphedema is a rare but recognized complication of Chlamydial infection (5, 21). While lymphangiosarcoma develops in long-standing lymphedema, e.g. following radical mastectomy for carcinoma of the breast (19), and occasionally in congenital or idiopathic lymphedema (12, 24), it has not been previously described following post-infectious lymphedema. Moreover, attempts to isolate organisms from Kaposi's sarcoma or to initiate the disease by passage of the tumor to animals or man have not been successful (1, 3, 9, 24). Recent studies (*W. Henle,* personal communication), however, are still vigorously pursuing a viral etiology.

Patients with chronic lymphedema of all varieties are particularly susceptible to secondary infection of the involved tissues. In addition, Chlamydial organisms are known to thrive in continuous tissue culture lines derived from malignancies (6). Therefore, it is conceivable that the Chlamydia isolated from our patient constitute an opportunistic infection in a patient with idiopathic lymphedema praecox. Nonetheless, the ease of its culture from a multitude of body fluids in this patient should stimulate the search for Chlamydial organisms in other patients with lymphedema or lymphangiosarcoma, as infection with this agent may be a cause rather than a result of these afflictions.

Table 1. Source and number of passages required for Chlamydial isolations obtained during the course of illness and at death

Source	Number of passages required		
	Yolks	Sac	3
Initial Study: Thoracic duct lymph	x		
Follow-up:			
1-2 months: Edema fluid from RLE*– and conjunctival fluid –			
Prostatic fluid and serum from RLE & LUE*		x	
Pleural fluid			x
4 months: Conjunctival fluid and serum		x	
7 months: Serum	x		
	McCoy Tissue Culture		
	1	2	
Post-mortem Examination:			
8 months:			
Tissues: Brain, scrotum, liver, heart, spleen, lung	x		
Inguinal and mesenteric lymph node, skin RLE		x	
Ascitic fluid and thoracic duct lymph		x	

*RLE (right lower extremity) and LUE (left upper extremity)

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M. Elvin-Lewis, Ph.D., Chairman, Department of Microbiology, School of Dentristry, Washington University, St. Louis, Missouri 63110/U.S.A.

Marlys Witte, M.D., Charles Witte, M.D., Department of Surgery, J. Davis, M.D., Department of Pathology, University of Arizona College of Medicine, Tucson, Arizona 85724/U.S.A.

W. Cole, M.D., Department of Surgery, University of Missouri School of Medicine, Columbia, Missouri 65201/U.S.A.

Lymphology 6 (1973) 121-12

The Effect of Arterio-Venous Fistula on the Lymphatics

K.G. Callum, J.B. Kinmonth*

Department of Surgery, St. Thomas's Hospital Medical School, London, S.E. 1, England

Summary

Arterio-venous fistulae were made in young growing pigs and lymphography was performed before and at regular intervals after operation. Animals were sacrificed at periods of two to six months. Small but consistent increases in limb growth resulted, but there was no change in the lymphatic vessels or nodes on lymphography or histological examination.

*Requests for reprints should be addressed to Professor J.B. Kinmonth, Department of Surgery, St. Thomas's Hospital Medical School, London, S.E. 1