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AIDS, KAPOSI SARCOMA, AND THE LYMPHATIC SYSTEM: UPDATE AND REFLECTIONS

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ABSTRACT

Based on ongoing basic and clinical investigations, further evidence is presented that the AIDS-Kaposi sarcoma (AIDS-KS) complex involves a progressive disturbance of the blood-lymph circulatory loop of fluid, macromolecules, and migrating cells. We first surveyed the spectrum of vascular abnormalties including Kaposi sarcoma (KS) found in the AIDS/ARC population, then non-invasively imaged lymphatic system abnormalities in AIDS-KS by whole body lymphangioscintigraphy, and finally examined the biologic behavior of AIDS-KS cells in long-term tissue culture. These observations are viewed in terms of the lymphatic and blood vascular route of entry and transport of free and cell-associated virus and other opportunistic pathogens as well as poorly understood host endothelial-immune system interactions.

During the symposium entitled "AIDS, Kaposi's Sarcoma, and the Lymphatic System: The Known and the Unknown" held at the XIth International Congress of Lymphology in Vienna in 1987, we proposed that AIDS, and particularly the AIDS-Kaposi sarcoma (AIDS-KS) complex, is a full-blown disorder of the integrated lymphatic system's four components--lymphatics, lymph, lymphocytes, and lymph nodes, which also form the battleground for the host response to the human immunodeficiency virus (1). Indeed, from what is already known, HIV is distinctly lymphotropic seeking out and destroying T-helper lymphocytes, the predominant circulating cell in lymph, and multiplying in antigen-presenting cells traversing the lymphatic system. This retrovirus invades and passes from host to host through a variety of tissue fluids or lymph and produces a local response (that is, lymph stasis) that interferes with the free movement of tissue fluid, macromolecules, and migrating cells, occasionally accompanied by overt lymphedema. Lymph nodes and other lymphoid aggregates are initially stimulated but finally exhausted in a process marked by intense neovascularization and vascular transformation, scarring, and obliteration and, in later stages, by aggressive lymphocyte-derived malignancies (lymphomas) and lymphatic-derived neoplasms like KS.

As the clinical syndrome unfolds, a striking resemblance emerges to a variety of congenital and acquired lymphologic syndromes characterized by one or more of the following features: lymph stasis, angiogenesis and fibrosis; depletion of immunocompetent cells and immunosuppression; opportunistic infections; and vascular neoplasms (2). Whereas lymph stasis of a body part, such as a lymphedematous leg, is associated with regional immunosuppression and opportunistic infections (e.g., repeated cellulitis) and vascular neoplasms (i.e., angiomas and even angiosarcomas as in Stewart-Treves

syndrome) thereby resembling a "local" form of AIDS, systemic immunodeficiency such as that induced by HIV may derive from a similar but more generalized lymphologic process accompanied by lymphocyte depletion, opportunistic infections, and widespread lymphatic vascular dysplasia and neoplasia (i.e., systemic AIDS).

Since the initial presentation of this speculative hypothesis on the pathogenesis of AIDS and Kaposi sarcoma (KS) in 1987, additional support has been gathered. First, other speakers at the Vienna symposium (namely, Ziegler, Dorfman, and Dictor) further implicated the lymphatic vasculature and specifically lymphatic endothelium in the genesis of KS lesions (3). In tissue sections of lymph nodes, Racz, Tenner-Racz, and Diebold identified circulating HIV+ lymphocytes in capsular lymph and infective virions on dendritic processes of antigen-presenting cells adjacent to germinal follicles (3). Barré-Sinoussi and Gottlieb emphasized more widespread dysregulation of resident and migrating lymphoid cell populations, and Ryan outlined the influence of physical and chemical factors such as subcutaneous edema in promoting endothelial migration and angiogenesis (3). Panelists discussed a variety of poorly understood immune system interactions with the vasculature and specifically, its endothelial lining.

Some original basic and clinical investigations since 1987 provide further insight into the lymphatic and blood vascular system's participation in AIDS and AIDS-related syndromes. We first surveyed the spectrum of vascular system involvement, including KS, found in the AIDS and ARC population, then noninvasively imaged lymphatic system abnormalities in AIDS-KS by whole-body lymphangioscintigraphy, and finally, examined the biologic behavior of AIDS-KS cells in long-term tissue culture.

First, what types of lymphatic and blood vascular lesions are seen in AIDS and ARC?

In 1981, the AIDS epidemic was ushered in by the dramatic appearance of an aggressive disseminated form of KS in young gay men in New York City focusing early attention on a curious link between vascular abnormalities and the immunodeficient state. Involvement of the mucocutaneous system is typically extensive in AIDS-KS *(Fig. 1),* including the face, trunk, extremities, oral cavity, genitalia, gastrointestinal tract, and lung, often accompanied by edema, serous effusions, bleeding, and, on rare occasions, by protein-losing enteropathy associated with lymphangiectasia.

Aside from KS, HIV-infected patients exhibit a bewildering array of vascular manifestations and exuberant angiogenesis that range from telangiectasia, lymphangiectasia, angiomas, angiofibromas, angiolipomas, pyogenic granulomas, and hepatic peliosis, to nodal vascular transformation, angioimmunoblastic lymphadenopathy (Castleman's disease), thrombophlebitis, vascular sclerosis, generalized purpura, leukoclastic vasculitis, and immune complex vasculitis *(Fig. 2)* (4-10).

The relationship of these and other vascular manifestations to circulating angiogenic factors generated directly by viral or other opportunistic infectious or indirectly by the host immune response is intriguing but poorly understood. For example, KS and other vascular lesions such as telangiectasias appear in other acquired and congenital immunodeficiency states and may disappear after immunorestoration. On the other hand, opportunistic invaders such as herpes zoster and simplex and cytomegalovirus (CMV), a variety of bacteria including mycobacteria, fungi, and protozoan parasites such as *Pneumocystis carinii* and *Toxoplasma gondii* promote vascular lesions that may regress with specific anti-microbial treatment such as chlamydia-induced epithelioid hemangioma, a KS "look-alike." Abuse of vasoactive recreational drugs, such as volatile amyl and butyl nitrite or "poppers," may also play a provocative or permissive role along with various genetic markers such as HLA determinants.

In this regard, it should be kept in mind that blood vessels and lymphatics,

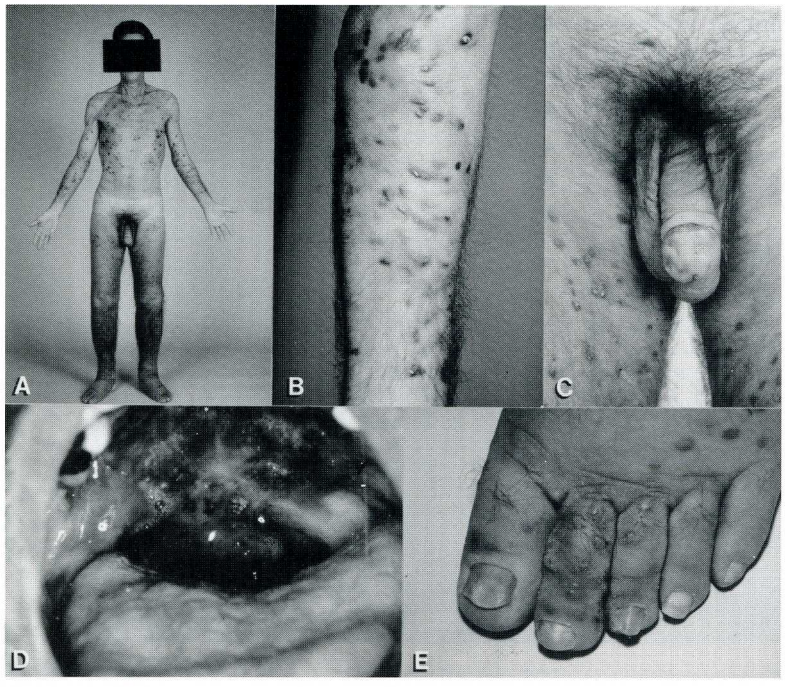

*Fig. 1. Extensive involvement of face (blocked out), trunk (**A**), extremities (**B,E**), oral cavity (**D**), and genitalia (**C**) in a patient with AIDS-associated Kaposi sarcoma. Note brawny lymphedema of both lower extremities. Characteristic constricting woody lymphedema also affects the left forearm but limb diameter is not obviously increased in this photograph.*

as pointed out in this symposium by Dr. Yoffey, are important points of entry, dissemination, and exit for invading microorganisms carried in host or foreign cells or as free particles, particularly when there are open sores as in sexually transmitted diseases. Endothelium and related reticuloendothelial cells or their stem cell precursors may also be directly infected by viruses releasing vasoactive cytokines that alter vascular permeability, basement membrane composition, and extracellular matrix formation. Furthermore, the cell-mediated and humoral immune reponse directed against a virus or other microbe, both in its protective and self-destructive aspects, takes place largely within the blood-lymph loop, where angiogenic and mitogenic substances are released and circulate through the extracellular fluid. Via the lymphatic system draining mucosal and skin surfaces, organisms and host products percolate slowly through regional lymph nodes before entering the bloodstream, first producing lymphoid stimulation and only later lymphoid cell drop-out and interstitial fibrosis. In intravenous drug abusers and HIV-infected transfusion recipients, hematogenous spread of the virus is direct. As Dr. Yoffey has described, endothelium takes up a variety of viruses at least in part by phagocytic mechanisms, and migrating macrophages and Langerhans cells, closely related to endothelium, are major sites of proliferation and transmission of viral particles through their extensive dendritic exterior surface area. Indeed, in acute experiments *in vitro*, herpes virus dramatically suppresses messenger RNA and DNA synthesis in endothelial cells thereby altering their cell surface, underlying basement membrane, and surrounding extracellular matrix, and perhaps even transforming the cells (11). Thus, the spectrum of vascular manifestations of

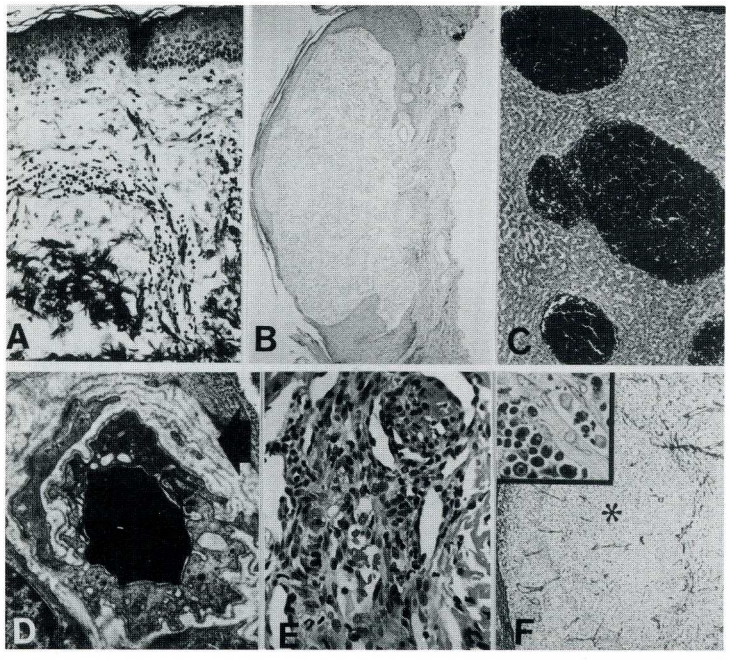

Fig. 2. Microscopy of vascular lesions in AIDS and AIDS-related syndromes. (A) Telangiectasia of the chest wall with intense perivascular lymphocytic infiltration around dilated capillaries (5). (B) Histiocytoid hemangioma showing epidermis forming a "collarette" that surrounds edematous stroma and contains increased number of vascular spaces (6). (C) Peliosis hepatis with randomly distributed blood-filled spaces often separated by only a few layers of hepatocytes (7). (D) Vascular sclerosis displaying, on this electron micrograph, extensive reduplication of basement membrane in a lymph node. Similar lesions occur in the heart, kidney, and other organs (8). (E) Early Kaposi lesion. There is a prominent infiltrate of lymphocytes and plasma cells interspersed among numerous irregular endothelial-lined vascular spaces in the reticular dermis (9). (F) Lymphadenitis with hypervascular follicular hyperplasia, a lesion spanning the spectrum between immunoblastic lymphadenopathy (Castleman's disease) and KS. There is extensive vascular proliferation penetrating altered germinal centers (see inset) with intense plasmacytic and immunoblastic overgrowth (10). (Reproduced with permission and modified into composite) (4).

AIDS raises intriguing questions about connections between the vascular and immune system, traceable back to a common embryonic anlage, and further, how viruses might trigger a widespread systemic disorder long after they are no longer detectable.

We next turned our attention to delineating the gross abnormalities in structure and function of the lymphatic system in AIDS and specifically AIDS-KS. Despite the distinctions often drawn between classical KS and endemic or African KS, on the one hand, and the epidemic form associated with AIDS on the other hand, Moritz Kaposi in his original treatise pointed out both the characteristic brawny lymphedema of the extremities as well as the widespread distribution of mucocutaneous lesions with occasional visceral involvement (12). Indeed, he ascribed a grim prognosis to KS. Subsequently, other workers noted the close resemblance of the irregular vascular channels making up the lesion to endothelial-lined lymphatics. Dictor and others at the Vienna symposium in 1987 (3), nonetheless, downplayed the morphologic and immunohistochemical distinctions between lymphatics and blood vessels proposing instead that KS evolves as anastomosing dedifferentiating radial veno-lymphatics. Still, the pathogenesis of lymphatic obstruction and lymphedema

in KS remains unclear, and mechanisms ranging from lymph node replacement by tumor to lymphatic sclerosis have been proposed (13).

Therefore, to delineate the anatomical and functional connection of KS lesions to the lymphatic system as well as the patterns in progressive disease, we performed whole body lymphangioscintigraphy in six HIV seropositive gay men with extensive cutaneous KS accompanied in four by visceral KS (14). 500μCi of technetium-labeled human serum albumin was injected intradermally into a web space on the dorsum of the foot or hand and serial images obtained using a wide field digital gamma camera capable of "sweeps" of the torso. The findings were compared to 30 other patients with lymphedema of different etiology and control subjects.

In normal limbs, peripheral and central lymphatic trunks were rapidly visualized, and uptake in regional inguinal or axillary lymph nodes was prompt. In contrast, in AIDS-KS patients, a variety of abnormalities was seen including numerous bulb-shaped "hot spots" filling from lymphatics in the distribution of cutaneous KS lesions, delayed tracer transport with absent, faint, or intense regional lymph nodal uptake, and retarded or impeded lymphatic drainage with intensification in the region of KS plaques *(Table 1)*. These findings document both an intimate connection between lymphatic trunks and KS lesions as well as impaired lymphatic transport and nodal dysfunction. Unlike a true primary neoplasm or metastatic malignancy, KS appears to arise as a budding multicentric endothelial proliferation primarily of lymphatics perhaps stimulated by retro- or co-viral infection directly, or indirectly by the host's immune response. Pathogenetic mechanisms underlying lymphedema in KS, operating singly or in combination in different patients or different limbs of the same patient, include opening of lymphatic-venous communications with bidirectional filling of lesions, lymphatic truncal sclerosis, proximal nodal obstruction from KS replacement or reticuloendothelial hyperplasia, and finally distal lymphatic obliteration from exuberant endothelial proliferation, intraluminal thrombosis, and tissue scar formation with or without repeated episodes of local infection producing features resembling lymphatic hypoplasia or aplasia *(Fig. 3)*. Whole body lymphangioscintigraphy may thereby assist in staging AIDS and KS (e.g., to delineate hyperplasia and later involution of lymph nodes) and assessing the degree of lymphatic blockage and interstitial fibrosis, as well as response to treatment including possibly regional endolymphatic therapy.

Table 1
Characteristic Lymphangioscintigraphic Features of AIDS-KS

- Delayed or absent lymphatic absorption of tracer from injection sites
- Early tracer entry into whole body blood pool
- Faint, distorted, or absent peripheral and central lymphatic images
- KS lesions "budding" from lymphatics
- Tracer pooling in KS lesions
- Faint, absent, or enhanced lymph node images

It is noteworthy that this experience was not our first in imaging lymphatic abnormalities in AIDS-KS. More than two decades ago in St. Louis in 1968, we encountered a black teenager with strikingly brawny bilateral leg and scrotal lymphedema who demonstrated total lymphatic obstruction at both groins after direct bipedal lymphography with poppy seed oil (15). After a progressive downhill course with worsening whole-body lymphedema, steeply declining peripheral blood lymphocyte counts, systemic chlamydial infection and marked wasting, autopsy showed widely disseminated Kaposi sarcoma. In our ignorance back then, we did not know what to call his disease. Not only did he retrospectively fulfill the CDC criteria for AIDS, but as reported in a preliminary fashion in the Vienna symposium (1) and subsequently further documented in collaboration with Drs.

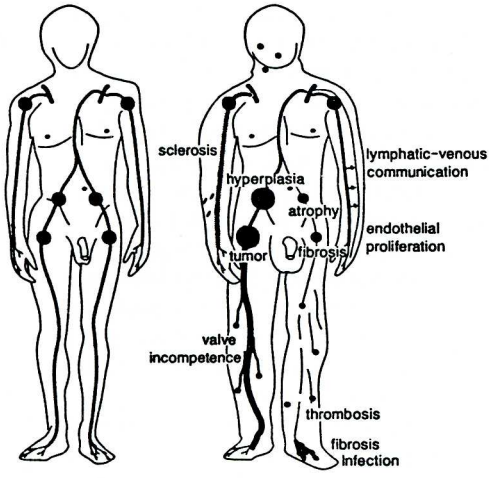

Fig. 3. Schematic diagram of pathogenetic mechanisms in AIDS-KS-associated lymphedema (right, compare with normal on left), operating singly or in combination in different patients or different limbs of the same patient: opening of lymphatic-venous communications with bidirectional filling of lesions (depicted schematically in the left upper extremity), lymphatic truncal sclerosis (in right arm), proximal nodal obstruction from KS replacement or reticuloendothelial hyperplasia (in right leg), and distal lymphatic obliteration from exuberant endothelial proliferation, intraluminal thrombosis, and tissue scar formation with or without repeated episodes of local infection producing features resembling lymphatic hypoplasia or aplasia (in left leg).

Gottlieb and Garry and our original St. Louis medical team (16), he was in fact infected with HIV-1 as shown by serum antibody to 9 of 9 HIV-1 antigens on Western blot immunoassay as well as HIV antigen by capture assay in preserved tissue.

Finally, in our latest studies we have moved from the previously described demographic survey of the spectrum of vascular lesions in the AIDS population and scintigraphic imaging of the lymphatic network in individual patients with AIDS-KS to the culture plate in an attempt to isolate and propagate AIDS-KS cells *in vitro*. KS cells have been notoriously difficult to grow long-term outside the body yet such a model system is needed to explore their biologic behavior in a controlled environment and to develop and test treatment modalities. What is causing the endothelial cells to proliferate and produce KS lesions in AIDS and can the process be inhibited or reversed? Several clues have been provided within the last year or two: Gallo and associates reported novel growth factors, produced by retrovirally-infected HTLV-II lymphocytes, which promote the growth of normal vascular endothelium and of cultured AIDS-KS cells derived from pleural effusions and pulmonary KS (17). These cultured AIDS-KS cells, which stimulate transient KS-like mouse tumors in immunodeficient nude mice (18) show distinctive immunohistochemical staining patterns presumably resembling lymphatic more closely than blood vascular endothelium. Furthermore, male but not female transgenic mice containing the HIV *tat* gene develop transient vascular tumors resembling KS (19). Nonetheless, the inability to demonstrate HIV in clinical KS or AIDS lesions and the rarity of KS in HIV infection acquired via the bloodborne route and in other retroviral infections weakens the direct link of KS to this retrovirus.

Based on our earlier success with long-term propagation of lymphatic endothelium from lymphangiomas (14,15), we prepared explant cultures of cutaneous KS lesions after excisional biopsy from five HIV seropositive gay males. Over several weeks to months, these primary cultures, which were not exposed to retrovirally conditioned media, were subjected to selective manipulations including modified enzymatic dissociation, variations in medium including altered serum concentration, short pulse trypsinization, hypoxia, and microcarrier beads for isolation of endothelial cell populations. Patterns on inverted light microscopy, transmission and scanning electron microscopy, and fluorescent Ulex ligand and Factor VIII-related antigen (F8AA) staining were used as endothelial phenotypic parameters. The findings were compared to tissue sections from the same patients as well as stock cultures of normal and neoplastic lymphatic and blood vascular endothelium. The cultured AIDS-KS cells

bear a strong physical and biologic similarities to their cells of origin on tissue section and closely resemble human lymphatic endothelial tumor cells *in vitro* but share features with blood vascular endothelium. They exhibit striking dendritic intercommunicating processes and transiently show cytopathic effects consistent with viral infection, although no specific virus has been detected. Based on immunohistochemical staining of coverslip preparations as well as flow cytometric analysis, most of the cells bear typical endothelial markers of F8AA and Ulex lectin, contain normal amounts of DNA and do not grow rapidly. Examination of factors regulating growth and organization of these cells is underway along with efforts to manipulate the process either by immunopotentiating drugs or angiostatic and angioinhibitory agents.

Finally, we are currently exploring further the intriguing link between angiogenesis (and specifically lymphangiogenesis) and lymphologic syndromes. Recently, we encountered a baby with systemic hemangiomatosis extensively involving the liver with cutaneous angiomas literally erupting daily, and subsequently, a young college student who presented with a large mediastinal cavernous lymphangioma mixed with venous hemangioma associated with hemangiomatous nodules studding the surface of his fibrotic lungs and accompanied by intermittent pleural effusion, peripheral edema, and a borderline low lymphocyte count. These angiogenic syndromes bear a striking resemblance to KS and, as Dictor (3) pointed out, to avian leukosis virus infection, which also exhibits exuberant angiogenesis and angiomas, and even angiosarcomas in a setting of general immune suppression (23). Recent studies suggest that the provirus for this infection of chickens inserts close to the epidermal growth factor gene (24). Can some of these benign and malignant angiogenic syndromes in man also be caused by retroviral infection, possibly detectable by such techniques as polymerase chain reaction?

Thus, in summary, during the intervening two years since the 11th International Congress of Lymphology, a body of information has emerged from a variety of sources confirming the widespread participation of the lymphatic system and the blood vasculature in AIDS and specifically, the AIDS-KS complex. Nonetheless, we are far from understanding the origin and evolution of this disease and particularly altering its fatal course. Accordingly, despite the explosion of fundamental knowledge about retroviruses and the immune system, the pandemic of AIDS is truly a case study in medical ignorance. Hand in hand with the tools of the molecular biologist, what is needed is the eye and the intellect of physician-investigators including lymphologists, to pose and explore questions about the pathophysiology, diagnosis, prognosis, and treatment of AIDS, KS, and related disorders of the blood-lymph loop.

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