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Short Communication

Extravasation and Transcytosis of Liposomes in Kaposi's Sarcoma-Like Dermal Lesions of Transgenic Mice Bearing the HIV Tat Gene

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Transgenic mice bearing the HIV tat gene develop dermal lesions resembling a common malignant tumor in AIDS, Kaposi's sarcoma (KS). To evaluate the permeability characteristics of these lesions and the therapeutic potential of drug-carrying liposomes, we have studied the localization of sterically stabilized liposomes, which show long circulation time in blood and increased accumulation in tumors. Liposomes encapsulating colloidal gold were injected intravenously into transgenic mice bearing KS lesions, and tissues were processed 24 hours later for both electron microscopy and for light microscopy with silver enhancement. Liposomes and silver marker were detected predominantly in the dermis surrounding the early and mature KS lesions, which were characterized by a proliferation of fibroblast-like spindle cells and abnormal blood vessels close to the epidermis. The silver-enhanced gold marker often surrounded vascular channels and scattered erythrocytes. As determined by electron microscopy, some spindle cells and macrophages had ingested intact liposomes. Transendothelial transport of liposomes was observed both through open channels between endothelial cells and also through endothelial cells lining intact vessels. Both extravasation and transcytosis of liposomes through irregular endothelium were much higher in KS lesions than in the adjacent normal skin. The high accumulation of sterically stabilized liposomes in KS lesions and their intracellular uptake by some spindle cells enhances their potential as carriers of chemotherapeutic agents against this neoplasm. (Am J Pathol 1993, 143:10–14)

Kaposi's sarcoma (KS) is a common malignant tumor in AIDS patients with a very poor prognosis.[1] Various studies have shown that the KS lesions are characterized by an increased permeability to blood components.[2,3] This makes liposome delivery of chemotherapeutic drugs to these lesions a very promising approach, especially because of the recent development of sterically stabilized liposomes[4–6] which have shown superior properties as drug carriers compared with conventional (nonstabilized) liposomes. Specifically, they have been shown to have a prolonged circulation time in blood and reduced uptake by the reticuloendothelial system,[6–8] as well as increased accumulation in implanted tumors.[4,8] More recently, they were also shown to have increased antitumor efficacy[4,9–12] and reduced toxicity for encapsulated antitumor drugs. In addition to the above results obtained in mice, promising results for chemotherapeutic drugs delivered by stabilized liposomes have been obtained in an ongoing clinical

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trial.[13] Preliminary pharmacokinetic studies comparing liposomal doxorubicin therapy to standard doxorubicin therapy in patients with AIDS-related KS suggest that the concentration of drug achieved within tumor tissue is increased by liposomal drug delivery.[14] The studies reported here suggest the mechanism by which the increased drug concentration within tumor tissue may be achieved: drug-containing liposomes extravasate through the discontinuous walls of vascular channels within KS lesions and accumulate in the tumor stroma.

Transgenic mice bearing the HIV tat gene develop dermal lesions resembling KS.[15] We have studied the localization of sterically stabilized liposomes in both early and late lesions in these mice in an attempt to clarify the mechanism of their accumulation in tumors and to enhance their potential as a drug carrier. For localization of liposomes at the cellular level, we have utilized encapsulated colloid gold as a microscopic marker. This newly developed method[16] provides us with an opportunity to follow the localization of liposome material both by electron microscopy and by light microscopy using silver enhancement.

Materials and Methods

The mice carried the LTR-tat sequence, which is a 2-kb KpnI-*Bfl*I fragment containing the complete transcriptional unit, purified from pHIV/LTR-tat3. The DNA fragment was microinjected into fertilized eggs from superovulated CD1 females (Charles River Laboratories, Bar Harbor, ME), which are outbred mice previously mated to (C57BL/6 × DBA/2) F_1 males (Jackson Laboratories, Wilmington, MD).

Liposomes composed of egg phosphatidylcholine (Avanti Biochemicals, Birmingham, AL), cholesterol (Sigma Chemical Co., St. Louis, MO) sterically stabilized with polyethylene glycol (M_r = 1900) conjugated to distearoylphosphatidylethanolamine at a 10:5:0.8 molar ratio were prepared by the method of reverse-phase evaporation with gold chloride/citrate in the aqueous phase.[16] After adjusting the pH and increasing the temperature, the majority of liposomes were 80 to 100 μ in diameter and contained one to three gold particles. Liposomes (approximately 0.2 ml, 2 μmol phospholipid) were injected intravenously via the tail vein into mice. Tissue samples were collected, fixed, and embedded in JB-4 resin 24 hours after injection. Thick sections were processed with silver enhancement solution (Amersham, Arlington Heights, IL) and then stained with hematoxylin and eosin.

Results

We investigated the localization of liposomes in both KS-like mature and early lesions, as well as in adjacent normal skin in the transgenic mice 24 hours after intravenous injection. By light microscopy with silver enhancement followed by hematoxylin and eosin staining, the lesions showed areas of hypercellularity with fibroblastic or spindle-shaped cells in the dermis (Figure 1A). In this region, the abnormal thickness of the papillary dermis, composed primarily of spindle-shaped cells with elongated cytoplasmic processes and oval nuclei, was apparent. This histological appearance is suggestive of the lesions of KS seen in patients with HIV infection.[2,3] Silver-enhanced colloidal gold particles were seen predominantly localized within the lesion in the region close to the epidermis. The dense concentration of silver particles was observed to surround abnormal blood vessels, scattered around nonendothelial bound streams of erythrocytes (Figure 1B). Some particles could be seen within the cytoplasm of some spindle-shape cells with elongated nuclei (Figure 1C).

Figure 1D shows the early lesions on the skin of a 8-month-old transgenic mouse. The lesions were predominantly composed of collagen. There were not as many spindle cells along the dermal-epidermal junction. The epidermal hyperplasia with accentuation of the granular layer and slight hyperkeratosis was observed. Silver-enhanced colloidal gold particles could still be seen to have crossed the blood vessel endothelium, extensively penetrating into the extravascular interstitial space between a few spindle-shaped cells. Figure 1E shows normal skin adjacent to the tumor. Its epidermis shows normal maturation and uniform thickness. There are very few cells in the dermal region. Only minimal silver staining can be seen here, primarily in and around the postcapillary venules. The silver deposits around the endothelium of blood vessels within the lesion (Figure 1B) is much denser and occurs more frequently than in adjacent normal skin (Figure 1E).

Examples of the distribution of colloidal gold-containing liposomes within the KS-like lesion at the ultrastructural level are shown in electron micrographs (Figure 2, A-D). Intact liposomes with gold particles could be seen in a large intracellular vesicle of a spindle cell (Figure 2A). Extravasated erythrocytes and liposomes (Figure 2B) are prominent, probably due to the irregular and discontinuously

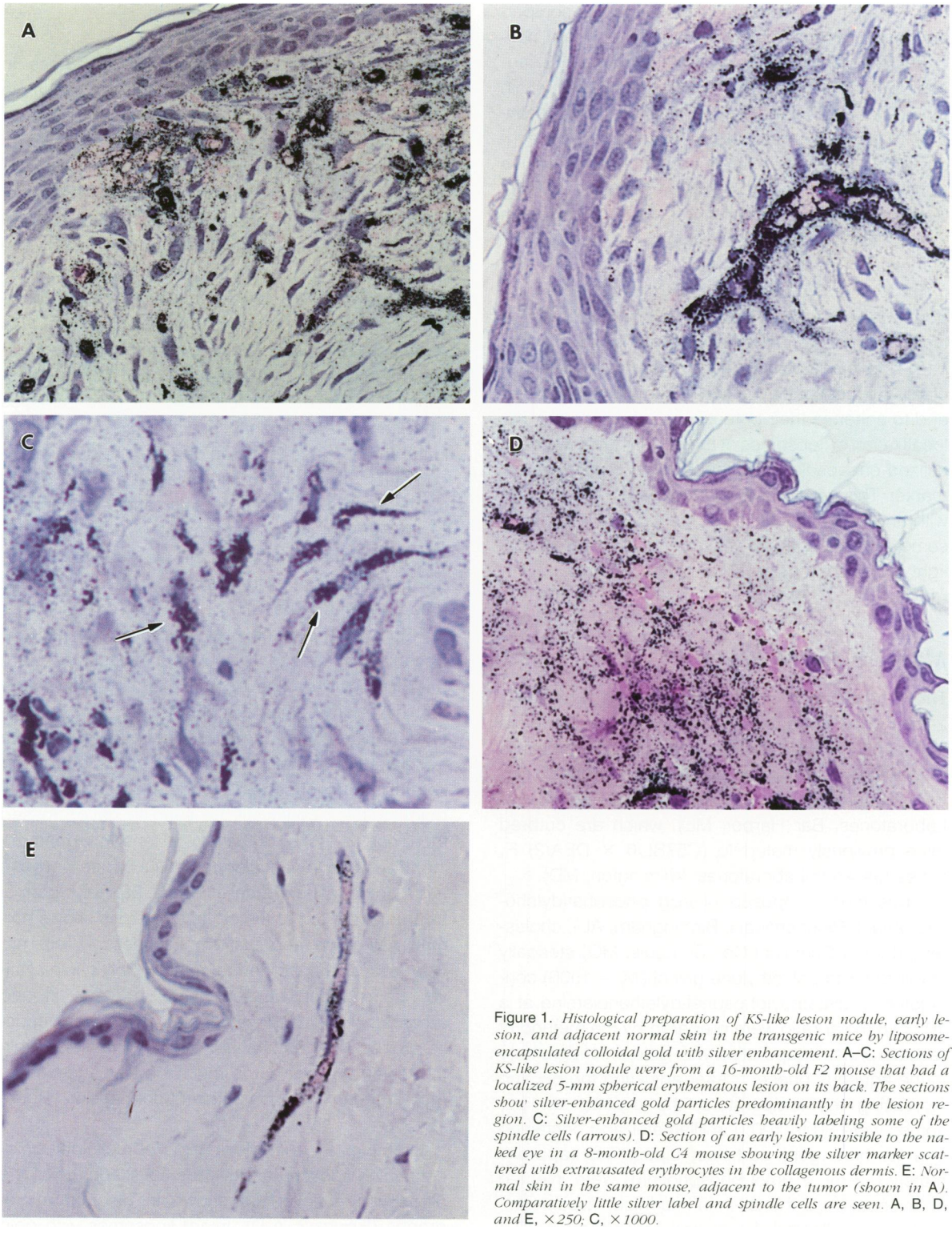

Figure 1. *Histological preparation of KS-like lesion nodule, early lesion, and adjacent normal skin in the transgenic mice by liposome-encapsulated colloidal gold with silver enhancement.* A–C: *Sections of KS-like lesion nodule were from a 16-month-old F2 mouse that had a localized 5-mm spherical erythematous lesion on its back. The sections show silver-enhanced gold particles predominantly in the lesion region.* C: *Silver-enhanced gold particles heavily labeling some of the spindle cells (arrows).* D: *Section of an early lesion invisible to the naked eye in a 8-month-old C4 mouse showing the silver marker scattered with extravasated erythrocytes in the collagenous dermis.* E: *Normal skin in the same mouse, adjacent to the tumor (shown in* A*). Comparatively little silver label and spindle cells are seen.* A, B, D, *and* E, *×250;* C, *×1000.*

lined endothelial channels. Gold particles also appeared in the endosomes and lysosomes of perivascular macrophages localized within the lesions (Figure 2C). Intact liposomes with colloidal gold particles could also be seen in vesicles, much larger than regular caveolae, across endothelial cells in lesions (Figure 2D) and presumably in postcapillary venules as well. These liposomes appear

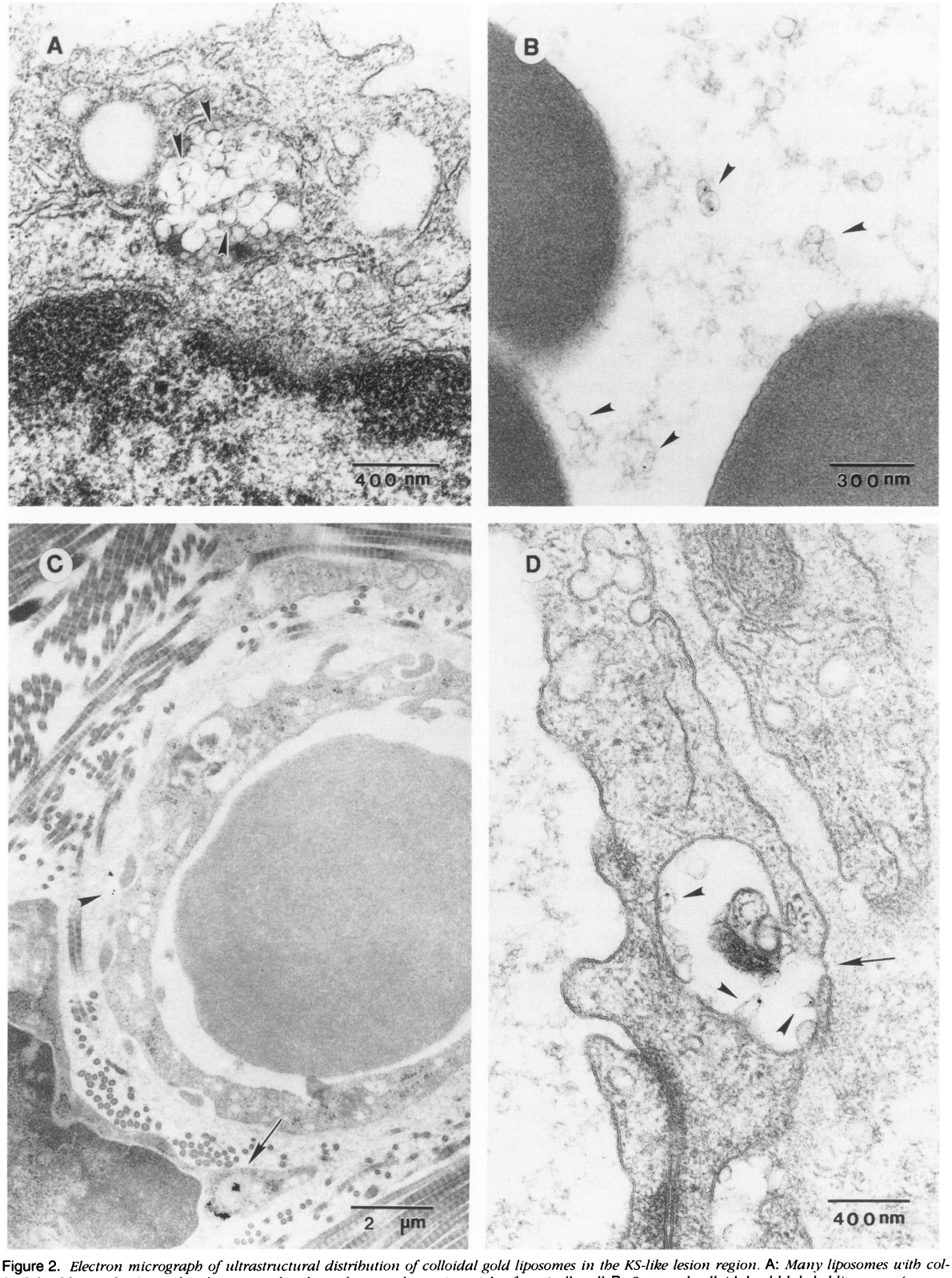

Figure 2. *Electron micrograph of ultrastructural distribution of colloidal gold liposomes in the KS-like lesion region.* **A:** *Many liposomes with colloidal gold particles (arrowheads) accumulated in a large endocytotic vesicle of a spindle cell.* **B:** *Scattered colloidal gold-labeled liposomes (arrowheads) near extravasated erythrocytes within the KS-like lesion.* **C:** *Gold particles accumulated in lysosomes (arrows) of a macrophage adjacent to a small blood vessel. The extravasation of liposomes with gold can also be seen in the endothelial cell basement membrane (arrowhead).* **D:** *Gold-labeled liposomes (arrowheads) in a large endothelial cell vesicle in the KS-like lesion. A fenestra (arrow) can be seen on the basement membrane side of the vessel. Notice that the tight junction is still intact, without dilatation of the extracellular space in some regions of the KS-like lesion.*

to be picked up from the blood vessel lumen and delivered to the extravascular space.

Discussion

Studies on the histogenesis of KS in AIDS patients have shown developing vessels often intermingled with spindle cells in the same lesion.[2,3] In the early stages of the lesion, an interlacing network of dilated vessels with irregular outlines appears. In the later stages of dermal tumor nodules, slit-like spaces containing erythrocytes are seen in the dermis, immediately underlying the hyperplastic epidermis.[1–3] These phenomena are seen not only in AIDS patients, but also in the LTR-tat transgenic mice. Although gold-labeled liposomes were found within the spindle cells which have a characteristically large and elongated nucleus (Figure 2A), we have not been able to further characterize these tumor-like cells. Histology of tissue sections for the tumor-like lesions of the transgenic mice indicated no reactivity toward the usual immune staining with antibody against human CD31, CD34, factor XIIIa, and proliferating cell nuclear antigen.

The inherent vascular leakiness observed here provides an advantage for the use of liposomes as a drug carrier in KS tumor lesions. The prolonged circulation time of sterically stabilized liposomes favors the opportunity for their extravasation through gaps between endothelial cells or openings at the neovascular termini during angiogenesis and accumulation. A second pathway for liposomes in the KS lesion is by a transendothelial route. The higher frequency of transendothelial vesicular transport is possibly due to the higher endocytic activity of the nascent endothelial cells during KS development. Due to the property of high accumulation of sterically stabilized liposomes in KS lesions and their uptake by some spindle cells, they appear highly attractive as carriers of chemotherapeutic agents for this neoplasm.

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