Kaposi’s Sarcoma in Renal Transplant Recipients

Ultrastructural and Immunoperoxidase Study of Four Cases

MOHAMMED AKHTAR, MD,* HERNANDO BUNUAN, MD,† MOHAMMED ASHRAF ALI, MD,‡ AND JOHN T. GODWIN, MD§

Tissues from four cases of Kaposi’s sarcoma developing in renal transplant recipients were studied by light and electron microscopic examination and by immunoperoxidase staining for Factor-VIII-related antigen. Ultrastructurally, the tumors in all four cases contained a variable mixture of cells, including endothelial cells, pericytes, fibroblasts, and myofibroblasts. These findings support the origin of Kaposi’s sarcoma from primitive vasoformative mesenchyme. Immunoperoxidase staining for Factor-VIII-related antigen was limited to endothelial cells. In one case intracytoplasmic virus-like tubular complexes were seen. The significance of this finding is briefly discussed.

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It is now well recognized that recipients of renal transplants may develop a variety of tumors including carcinoma, sarcoma, and lymphoma. Approximately 5% of these tumors are Kaposi’s sarcoma.1,2

At King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia, a total of 51 renal transplant patients have been followed over a 7-year period extending from July 1975 to June 1982. Of these, there were four malignant neoplasms, all of which were Kaposi’s sarcoma. Five biopsy specimens taken from the patients were studied by light and electron microscopy and by immunoperoxidase staining for Factor VIII antigen (Ag). We record our findings in these cases to clarify the morphogenesis and histogenesis of Kaposi’s sarcoma.

Materials and Methods

The salient clinical features of the four cases are presented in Table 1. For light microscopic examination, the tissues were fixed in 10% neutral buffered formalin solution and embedded in paraffin. Four-micron-thick sections were stained with hematoxylin and eosin stain (H & E). For electron microscopic examination the biopsy material was fixed in 3 glutaraldehyde buffered in Millonig’s phosphate buffer (pH 7.3), postfixed in 1% osmium tetroxide, dehydrated through graded acetones and embedded in Spur. One-micron-thick sections were stained with toluidine blue for orientation. Ultrathin sections were stained with uranyl acetate and lead citrate prior to examination by the electron microscope.

For immunohistochemical localization of Factor VIII Ag five-micron-thick sections of formalin-fixed and paraffin-embedded tissue were processed in the following order using reagents from DAKO PAP Kit (DAKO Corporation, Santa Barbara, CA): (1) Sections were deparaffinized in xylene and hydrated in graded alcohols. (2) Endogenous peroxidase activity was blocked with a 5-minute incubation in 3% H2O2. (3) After rinsing with tris-buffered saline, the slides were incubated with normal swine serum for 20 minutes. (4) The sections were then incubated with rabbit antihuman Factor VIII Ag for 20 minutes. (5) After rinsing with tris-buffered saline, the slides were incubated with normal swine serum for 20 minutes. (6) After rinsing with tris buffer slides were incubated with peroxidase-antiperoxidase (PAP) reagent for 20 minutes. (7) After rinsing with tris buffer slides were incubated with peroxidase-antiperoxidase (PAP) reagent for 20 minutes. (8) After rinsing with distilled water, sections were counterstained with Mayer’s hematoxylin.
TABLE 1. Salient Clinical Features of the Three Patients with Kaposi's Sarcoma Developing Following Renal Transplantation

| Case no. | Age/sex | Type of graft   | Interval between transplantation & KS (mo) | Location of lesions                  | Type of immunosuppressive therapy | Management, follow-up                                                                 |
|----------|---------|----------------|------------------------------------------|--------------------------------------|-----------------------------------|---------------------------------------------------------------------------------------|
| 1        | 43/M    | Living related donor | 13                                       | Both thighs, left hand, chest wall, oropharynx | Imuran & prednisone               | Doses of Imuran & prednisone reduced. Lesions treated with local radiation. Patient doing well 3 years later. |
| 2        | 20/M    | Cadaver         | 4                                        | Both lower extremities               | Imuran & prednisone               | Doses of Imuran & prednisone reduced. Lesions treated by local radiation. Patient died with thrombocytopenia, leukopenia & bronchopneumonia after 3 wk. |
| 3        | 36/F    | Living related donor | 12                                       | All four extremities                 | Imuran & prednisone               | Doses of Imuran & prednisone reduced. The disease was controlled, but the older lesions persisted. |
| 4        | 54/M    | Cadaver         | 4                                        | Left forearm, both ears, legs and thighs, left thumb. | Cyclosporin, 600 mg/d, prednisone, 20 mg/d | Doses of cyclosporin and prednisone reduced to 100 mg and 10 mg/d, respectively. Lesions regressed. Biopsy specimen taken 3 months later showed persistent KS. |

KS: Kaposi's sarcoma.

Results

Light Microscopic Findings

The light microscopic findings from all four patients were essentially similar. The tumor, involved the dermis and subcutaneous adipose tissue, and were composed of proliferated intertwining bundles of spindle cells. In addition, several well-formed vascular spaces were scattered between the tumor cells (Fig. 1). Extravasation of erythrocytes in the interstitial spaces and aggregates of he-

![Fig. 1. Case 2. Photomicrograph featuring a typical area of the tumor. There are intertwining bundles of spindle-shaped cells interspersed with endothelial-lined vascular spaces (H & E, original magnification ×300).](image-url)
FIG. 2. Case 2. A low-magnification electron micrograph depicting a typical area of the KS. Two vascular spaces (VL) lined by endothelial cells are seen. The area between these vascular spaces is occupied by stromal cells which are irregularly shaped and are separated from one another by collagen fibers (uranyl acetate and lead citrate stain, original magnification \( \times 7000 \)).

FIG. 3. Case 3. A higher magnification electron micrograph depicting portion of a blood vessel wall. The vessel is surrounded by a pericyte which is partly covered externally by basal lamina (larger arrows). The cytoplasm of the pericyte contains several profiles of rough endoplasmic reticular and focal accumulation of myofilaments with densities (small arrows). Several pinocytic vesicles are also noted (uranyl acetate and lead citrate stain, \( \times 12,000 \)).
mosiderin pigment were also frequent. Occasionally, small clusters of inflammatory cells including lymphocytes and plasma cells were present.

**Electron Microscopic Findings**

The ultrastructural appearance of the tumors from all four cases was essentially similar. The lesion were composed of two components, vascular spaces and stromal cells (Fig. 2).

**Vascular spaces:** These were of variable size, and were essentially composed of a layer of endothelial cells surrounded by basal lamina. The lumen varied from a slit-like space to a widely patent space containing erythrocytes and leukocytes. The endothelial lining was not fenestrated. Some of these spaces were also surrounded by one or more layers of pericytes. These pericytes varied considerably in the degree of differentiation. Some had an appearance typical of pericytes characterized by the presence of large numbers of filaments with densities, numerous pinocytic vesicles, and a well-developed basal lamina. Others were less differentiated and had prominent cisterns of rough endoplasmic reticulum and fewer numbers of cytoplasmic filaments and pinocytic vesicles (Fig. 3).

**Stromal cells:** These were present in the spaces between the well-formed vascular spaces and also in areas of the tumor where vascular spaces were less prominent. A variety of cells was present in these areas; these included the following: endothelial cells, pericytes, fibroblasts, myofibroblasts, and undifferentiated cells. Endothelial cells were recognized by intercellular junctions, and were partly surrounded by a basal lamina (Fig. 4). Usually no definite lumen was discernible, although occasional erythrocytes were present among these cells. Pericytic cells were usually present in close proximity to the endothelial cells, although occasionally they were seen without accompanying endothelial cells.

Myofibroblasts were scattered randomly within the stromal areas. These were characterized by the presence of prominent cisterns of rough endoplasmic reticulum. In addition, prominent bundles of fine filaments were present immediately adjacent to the plasma membrane (Fig. 5). A few pinocytic vesicles were also present.

Fibroblasts were irregular in configuration and recognized by the presence of distended cisterns of rough endoplasmic reticulum containing finely granular material. Cytoplasmic filaments were scanty (Fig. 6).
Undifferentiated cells: These were round to irregular in configuration. The cytoplasm contained a few organelles, such as mitochondria and occasional profiles of rough endoplasmic reticulum (Fig. 7). No cytoplasmic filaments or pinocytic vesicles were present. Lysosomes were occasionally present.

Scattered among the cells in the stromal areas were variable amounts of elastic and collagen fibers. In addition, in several areas extravasated erythrocytes were present in the intercellular spaces. Occasional tumor cells contained intracytoplasmic bodies resembling fragments of erythrocytes (Fig. 8).

In the biopsy specimen from Case 4 some of the tumor cells contained intracytoplasmic aggregates of twisted, branching tubular structures. These were present mostly within the endothelial and perithelial cells. The tubules were approximately 35 nm in diameter (Fig. 9). Smaller aggregates were easily recognizable as arising within cisterns of endoplasmic reticulum; however, a clear relationship with the endoplasmic reticulum was not always discernible in larger aggregates. A repeat biopsy from this patient 3 months after reduction of immunosuppressive therapy did not show such tubular complexes within the tumor cells.

Immunohistochemistry

Tissues from all four patients were positive for Factor VIII Ag. The reaction was most pronounced in the cytoplasm of the endothelial cells lining the vascular spaces (Fig. 10). Within the stroma, occasional isolated cells were also positive, but there was no positive reaction in large areas of stromal zones.

Discussion

The histologic appearance of Kaposi's sarcoma (KS) is quite characteristic in the majority of cases so the histopathologic diagnosis is usually not difficult. The tumor is typically composed of a variable mixture of endothelial-lined vascular spaces and spindle-shaped cells. In addition, extravasated erythrocytes and clusters of inflammatory cells including lymphocytes and plasma cells may also
be seen. Occasionally it may be composed entirely of the spindle-shaped stromal cells with virtual exclusion of vascular spaces. The diagnosis of KS in these cases may be extremely difficult.

Ultrastructure of KS has now been described in several reports. These have demonstrated a variety of mesenchymal cell types such as fibroblasts, endothelial cells, pericytes, histiocytes, smooth muscle cells, and myofibroblasts (Table 2). In one study, the tumor cells were categorized as Schwann cells on the basis of the presence of intracytoplasmic lamellated structures resembling myelin sheaths, but these findings have not been confirmed by subsequent studies. These lamellar inclusion bodies are best interpreted as residual bodies of lysosomes. In the remaining studies the fibroblast appears to be the cell which has been identified by most observers, followed by the endothelial cell and perithelial cell. In one study, no pericytes were identified, although cells with smooth muscle differentiation were noted. We believe that these are more appropriately interpreted as pericytes rather than smooth muscle cells.

Our ultrastructural studies indicate that the blood vessels in KS are usually composed of a single layer of endothelial cells which may also be surrounded by a layer of pericytes. Within the intercapillary spaces the cells are elongated or irregular in configuration. These manifest varying stages of formation of blood vessels as indicated by the presence of cells differentiating into endothelial and perithelial cells. Some of the cells in these areas resemble fibroblasts, while others have the structure of myofibroblasts. In addition, in some of the cells the differ-

FIG. 8. Case I. Electron micrograph of an endothelial cell (arrow) containing electron-dense rounded bodies within the cytoplasm resembling fragments of erythrocytes (uranyl acetate and lead citrate stain. original magnification X6000).

FIG. 9. Case 4. Electron micrograph featuring portion of a pericyte containing cytoplasmic tubular complexes (arrow) (uranyl) acetate and lead citrate stain. original magnification X15,000).
entiation is rather poor, so that no exact categorization can be made.

The histogenesis of KS is still controversial. Several hypotheses have been advanced with regard to the cell of origin. Pepler and Theron suggested an origin from Schwann cells on the basis of the presence of intracytoplasmic lamellated bodies which they interpreted as resembling the myelin sheath. These findings, however, have not been confirmed in subsequent studies. Niemi and Maskatellio suggested that the tumor is derived from pericytes, whereas Hashimoto and Lever concluded that the tumor is composed of two kinds of cells, namely endothelial and perithelial cells. Harrison and Kahn proposed an origin from primitive mesenchymal cells which have the potential to differentiate into several specialized connective tissue cell types. We believe that KS is derived from primitive vasoformative mesenchyme.

The demonstration of myofibroblasts in our cases of KS, as well as in one previous report by Harrison and Kahn, is of interest. Myofibroblasts may be seen in a variety of soft tissue tumors, such as malignant fibrous histiocytoma, fibrosarcoma and fibromatosis. This cell is also known to form a major component of the spindle cells in granulation tissue. In granulation tissue there is proliferation of endothelial-lined spaces which are separated from one another by spindle-shaped cells. This structural arrangement is identical to that seen in KS.

Ultrastructural studies of granulation tissue have revealed that the vascular spaces are lined by endothelial cells which are surrounded externally by well-developed basal lamina; these in turn may be surrounded by a layer of pericytes. The cells present within the intercapillary spaces have the ultrastructural features of fibroblasts and myofibroblasts. Thus, it is apparent that granulation tissue resembles KS not only in its morphologic appearance, but also in the type of cells present. It may therefore be speculated that KS represents the malignant counterpart of granulation tissue.

Immunoperoxidase staining for Factor VIII Ag has been shown to be a reliable method for identification of endothelial cells. In our four cases immunoperoxidase studies for Factor VIII Ag demonstrated the presence of this antigen primarily in the endothelial cells (Fig. 10). The spindle cells in the stroma were mostly negative, except for occasional cells which showed slight positivity. This would indicate that in KS endothelial cells represent

| Cell types | Endothelial | Perithelial | Fibroblastic | Others | Comments |
|------------|-------------|-------------|--------------|--------|----------|
| Pepler and Theron, 1962 | - | - | - | Schwann cell | The interpretation of tumor cells as Schwann cells was based on the demonstration of lamellated structures thought to represent myelin sheaths. These are best interpreted as residual bodies of lysosomes. |
| Hashimoto and Lever, 1964 | + | + | + | Histioyte | 
| Niemi and Mustakallio, 1965 | - | - | + | Mast cells | 
| Motaz and Zelickson, 1966 | + | + | + | Reticular mesenchyme | 
| Martinez-Penuela et al., 1970 | - | - | + | Smooth muscle myofibroblasts | No perithelial cells were recognized. Presumably the cells with apparent smooth muscle differentiation represented pericytes. |
| Harrison and Kahn, 1978 | + | - | + | Myofibroblasts |

| Akhtar et al. | + | + | + | 

*Fig. 10. Case 3. Photomicrograph of an area from KS stained by immunoperoxidase technique for Factor VIII Ag. Small vascular spaces with positive reaction within the cytoplasm of endothelial cells can be seen. Stromal cells are mostly negative (original magnification X85).
only one of several distinct types of mesenchymal cells. These findings lend further support to the concept that KS, although a vascular tumor, is not of pure endothelial origin but is derived from another more pluripotential cell which can differentiate into a variety of other cell types besides the endothelial cell.

In a recent study of 37 cases of KS by immunoperoxidase staining for Factor VIII Ag, Nadji and associates found a positive reaction in all their cases regardless of their histologic variations.14 Although the intensity of reaction was greater in cells that lined vascular channels and blood filled clefts, many intertwined spindle cells also contained Factor VIII Ag in their cytoplasm. They interpreted these findings to indicate that KS is of endothelial derivation.

The immunohistochemical findings in our four cases are at variance with those of Nadji and associates14 because in our cases the reaction of Factor VIII Ag was essentially limited to endothelial-lined spaces, and staining of the stromal cells was infrequent. The reason for this apparent disparity is not clear. It may be that some of the cells in the stroma which are beginning to differentiate into endothelial cells are responsible for the positive reaction.

The demonstration of cytoplasmic tubular complexes (CTC) within the tumor cells in Case four merits further comment (Fig. 9). Similar tubular complexes have been encountered in neoplastic processes including leukemia, lymphoma, carcinoma, malignant melanoma, and osteosarcoma.15-19 Also, similar aggregates have been observed in lymphoreticular and endothelial cells from patients with collagen-vascular diseases and immune deficiency states.20 The names applied to these structures include tubuloreticular complexes, tubular structures, tubular complexes of endoplasmic reticulum and virus-like particles; we prefer to use the term cytoplasmic tubular complexes (CTC).

The significance and pathogenesis of the CTC are still not certain. Many authors have emphasized the similarity of CTC to virus particles. Cells infected by virus initially show a proliferation of endoplasmic reticulum and of Golgi apparatus with subsequent appearance of virus particles and of membranous or tubular masses in the cytoplasm.21,22 The morphologic similarity between the tubular aggregates seen in virus-infected cells and CTC seen in various tumors and autoimmune diseases is quite striking; however, significant ultrastructural differences have also been noted, raising doubts about a viral origin of these complexes.20 A nonviral origin is also supported by the fact that these complexes have been produced in vitro in the human lymphoma cell line following an S-phase exposure to halogenated pyrimidines apparently in absence of any virus.23

Case 4 in which CTC were encountered was different from the other three cases because in this patient cyclosporin was used as an immunosuppressive agent rather than prednisone and Imuran. Whether or not the formation of CTC in our patient was in any way related to cyclosporin therapy is not clear. It may be of interest to note, however, that these complexes could not be demonstrated in the second biopsy which was performed 3 months following reduction of the cyclosporin dose. The effect in humans of prior treatment by chemotherapeutic or immunosuppressive agents on the development of CTC is unknown. Similar tubular complexes have been observed in renal tubular epithelial cells in animals receiving vinblastine.24 Thus, it is quite possible that the development of CTC in our case was related to cyclosporin therapy; however, experience with additional cases treated in this fashion is needed before a definite cause-and-effect relationship can be established.

The four cases of KS described in this report were seen in a group of 51 renal transplant recipients followed at King Faisal Specialist Hospital and Research Centre from July 1975 to June 1982. During this period a total of 11 cases of KS were also seen in the general patient population at this institution. The light microscopic appearance of these tumors was identical to that of the transplant group. Three of these biopsy specimens were further studied by electron microscopy, and, again, no significant morphologic differences were noted between the two groups. None of the biopsy specimens revealed CTC within the tumor cells.

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