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Spindle Cell Ploidy and Proliferation in Endemic and Epidemic African Kaposi's Sarcoma

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Comparative studies of ploidy and proliferative activity of spindle cells in sections of 20 (skin, 17; lymph node, 3) biopsy specimens from African patients, 10 with endemic Kaposi's sarcoma (EKS) and 10 with AIDS-associated Kaposi's sarcoma (AKS) were performed by histopathology, feulgen-based DNA measurement and proliferating cell nuclear antigen (PCNA)/cyclin immunohistochemistry, respectively. All specimens were classified as nodular lesions with basically the same histology. In 17 cases immunostained for cyclin/PCNA, the percentage of proliferating spindle cells range between 2–18, with a higher mean rate in AKS although this was not statistically significant. *In situ* measurement of DNA showed no significant values greater than the diploid level of control cells indicating that spindle cells in both EKS and AKS have euploid DNA content. Our findings indicate that both EKS and AKS represent the same type of euploid low rate cell proliferations. This corroborates previous suggestions that KS could represent a reactive process to yet undefined stimulus rather than a clonal proliferation, of transformed malignant cells.

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INTRODUCTION

THE NATURE and origin of the histologically complex, tumour like lesion known as Kaposi's sarcoma (KS) is controversial [1]. It presents in four distinct clinico-pathological forms: classical KS (CKS), the endemic African KS (EKS), KS associated with the acquired immunodeficiency syndrome (AKS), and the iatrogenic form developing in immunosuppressed patients undergoing organ transplantation (IKS) [2–5].

EKS is seen both in adults and children in East and Central Africa with an adult male to female ratio of 10:1 which is less pronounced in children. AKS is more often seen in young to middle-aged individuals. From epidemiological data AKS appears to be more often associated with homosexual then with parenteral transmission [6]. Adult EKS usually presents with indolent lesions confined to the extremities and has generally a better prognosis [2, 3] than AKS which is usually disseminated with extensive skin, lymphoglandular and visceral lesions and has a rapid clinical course similar to paediatric EKS [3–5].

The virtually identical histopathology of the various KS forms suggest a common pathogenic mechanism(s). However, distinct pathogenic mechanisms cannot be ruled out [6].

The spindle cells of KS are generally believed to be endothelial derived [7], but their lymphatic [8] or vascular origin [9] is still controversial. Furthermore in a recent study we have shown that the spindle cell compartment of KS is heterogeneous (unpublished data). The neoplastic nature of KS has been regularly questioned although clinically it behaves like a tumour [1, 10, 11]. Recent *in vitro* studies of cultured KS cells also seem to support the hypothesis that KS may represent a reactive lesion [12–15]. Measurement of DNA directly in histological sections has allowed the assessment of tumour ploidy and its correlation to prognosis [16, 17]. Also recently antibodies to proliferating cell nuclear antigen (PCNA)/cyclin which is an auxiliary protein of DNA polymerase delta has made it possible to define by immunohistochemistry cells in the late G1, S and G2/M phases of the cell cycle. Maximum labelling is observed in the S phase, correlating directly with cellular proliferation [18, 19]. Thus combination of DNA morphometry for ploidy evaluation and immunohistochemistry for determination of cell proliferation provides objective criteria allowing a more biological and functional definition as to the nature of the proliferating cells in KS [16].

Here we have combined these assays in a comparative study of spindle cells in EKS and AKS.

MATERIALS AND METHODS

20 cases (10 EKS and 10 AKS) of KS obtained from Muhimbili Medical Centre, Tanzania, formed the basis of this study. HIV serology was performed by ELISA and confirmed by western blot [20]. Clinical data of the patients and results of serology are shown in Table 1.

Paraffin sections

Skin or lymphnode biopsy samples were partly fixed in 10% formalin and partly in Carnoy's fixatives. Paraffin embedding was done and parallel sections were made from the blocks. One section was stained routinely with haematoxylin and eosin and two succeeding sections were used for cyclin immunohistochemistry and DNA measurements.

Immunohistochemistry

Paraffin embedded sections from 17 cases were deparaffinised in an oven at 60°C for 50 min, then in xylene at 60°C for 10 min and two changes of xylene, for 10 min each. Rehydration was done by graded ethanol to water. Endogenous peroxidase activity

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Table 1. Characteristics of nodular African endemic and epidemic Kaposi's sarcoma

No.	Age	Sex	HIV status	Site and extension	Cyclin, % Positive cells
1	30	M	–	Right arm nodules	NT
2	38	M	–	Skin lesions in the extremities	NT
3	36	M	–	Skin lesions	NT
4	58	M	–	Multiple skin nodules	5
5	83	M	–	Nodular skin lesions, lower limbs	17
6	56	M	–	Nodular skin lesions	10
7	69	M	–	Skin nodules	5
8	26	M	–	Skin nodules	14
9	70	M	–	Skin nodules	4
10	31	M	–	Skin nodules	10
11	32	M	+	Generalised skin lesions	5
12	28	M	+	Generalised skin lesions	18
13	30	M	+	Nodular skin lesions	17
14	Adult	M	+	Generalised, skin, lymph nodes	15
15	34	M	+	Generalised, skin, lymph nodes	12
16	34	M	+	Skin nodules	9
17	34	M	+	Skin nodules	2
18	24	F	+	Skin nodules	18
19	26	F	+	Skin nodules	15
20	20	F	+	Generalised, lymph nodes, oral	3

NT = Not tested due to inadequate material.

was blocked by passing the sections in 5% hydrogen peroxide in methanol for 25 min. The sections were then incubated over night at 4°C with anti-proliferating cell nuclear antigens Mab (PCNA)/cyclin, clone 19F4 (Boehringer Mannheim Biochemicals) or NCL-PCNA, clone PC10 (Novocastra Laboratories, UK). Peroxidase anti-peroxidase (PAP) and avidin biotin peroxidase complex (ABC) methods were used as previously described [21]. Positive controls consisted of sections of human tonsil or lymphnode germinal centres. In lymphnodular KS remaining lymphoid germinal centres were used as internal positive controls. Negative control was done by substituting anti-cyclin with TBS. Slides were then lightly counterstained by Harri's haematoxylin, briefly dehydrated in alcohol to xylene and mounted with eukit. Evaluation of cyclin (PCNA) immunohistochemistry was done on sections microscopically (Nikon Labophot/HFX microscope), in 10 high power (X63 objective) microscopic fields, and an eye piece fitted with a graticule. Approximately 1000 cells were counted. PCNA index was calculated as: no. of PCNA positive spindle cells per total no. of counted spindle cells, expressed as a percentage. A positive reaction was defined as a clearly visible, weak to strong immunostaining of the cell nucleus either granular and/or diffuse (Fig. 1).

DNA measurement

Paraffin sections from 9 cases were dewaxed and stained by Feulgen's method as described previously [22]. Representative fields of the tumours in the Feulgen stained preparation were selected and pictures taken in a Leitz photomicroscope with a 40× objective using a Kodak technical pan film (Technidol Photo-Flo). The developing time was standardised to 4 min at 22°C.

The cytometric method used in this study is based on photometric evaluation of the light transmission of the Feulgen stained (DNA) nuclei, and is a modification [22] of Adam's original description [23]. Approximately 100 tumour cell nuclei were measured on each specimen. Individual tumour cells were selected at random within the photographed area on the basis of morphology and measured as described previously [24]. The modal DNA value (MV) of the KS cells analysed were calculated as previously described [24], in relation to the DNA (2C) of normal cells outside the tumour area which provided internal control. DNA content was expressed in C units, 2C being defined from the median (P50) of the control cells. To distinguish non-diploid from diploid cell populations an upper limit of 2.5C was set for the diploid value. This value was empirically selected as not being exceeded by most (90%) of the control cells. The percentage of KS cells above 2.5C (2.5C exceeding rate) limit was taken as a measure of non-diploid. However, since the 2.5C exceeding rate also contains proliferating diploid cells the percentage of cells with DNA content exceeding the non-diploid cells or aneuploidy was set at 5C (5C exceeding rate). The 5C exceeding rate is defined as the percentage of cells whose nuclei contain more than five chromosomes [25].

RESULTS

Patients' data, HIV status and percentage of cyclin/(PCNA) expression of the biopsy specimens are summarised in Table 1.

Histopathology

No major differences were noted between endemic and AIDS associated KS, with regard to histology. All biopsy specimens were classified as characteristic nodular lesions (Fig. 2). In the skin the lesions were located within the upper or middle dermis. Lymph node lesions involved most of the node with extensive destruction of architecture but in no case was extranodular infiltration observed. In both skin and lymph node lesions most cells were interlacing consisting of a predominantly spindle cell population intermixed with vascular slits and extravasated erythrocytes. Lymphocytic and plasma cell infiltrate were rare to moderate within the spindle cell compartment. In marginal areas of the KS lesions the vessels were dilated and sometimes had a jagged appearance.

Immunohistochemistry

Cyclin (PCNA) expression varied between 2 and 18% of the spindle cells in the 17 cases (Table 1). The immune reaction for

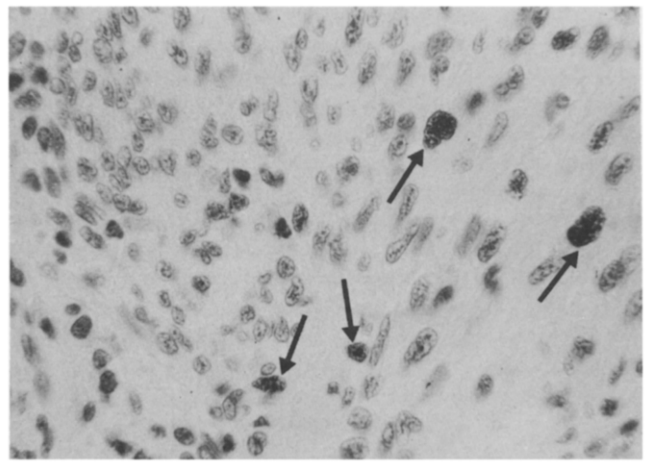

Fig. 1. Immunostained KS lesion showing several PCNA positive (arrow) spindle cells (× 600).

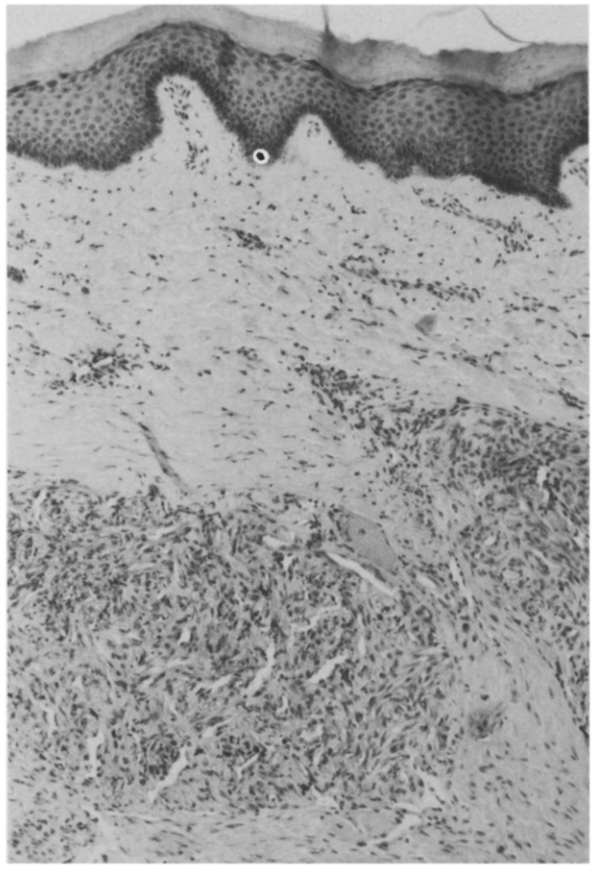

Fig. 2. Histological section of a nodular KS lesion showing interlacing spindle cell proliferation and vascular slits (haematoxylin and eosin × 160).

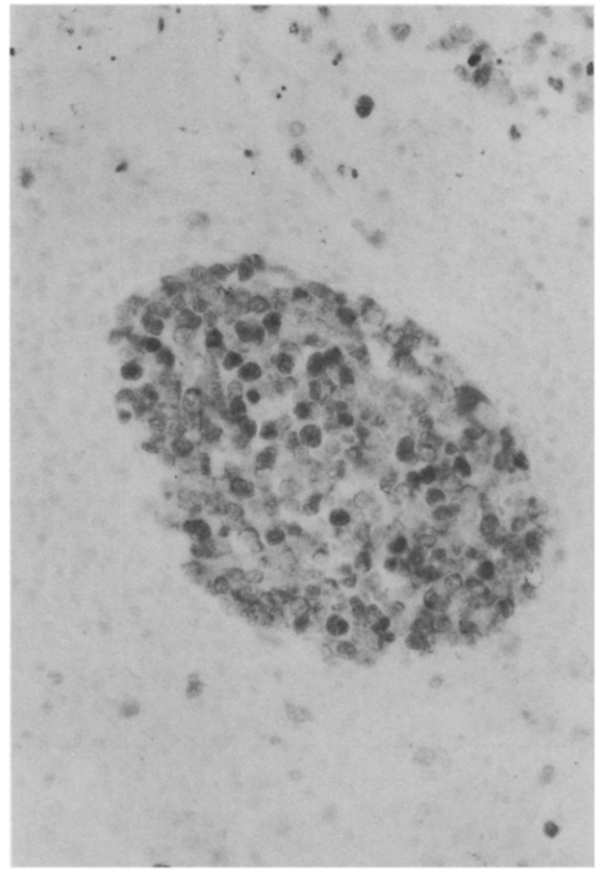

Fig. 3. Immunostained lymphnode section showing extensive staining of germinal centre cell with PCNA (× 400).

cyclin was exclusively localised to the nuclei (Fig. 1). The staining pattern was diffuse or mixed diffuse and granular. The intensity of the immune reaction varied but was usually distinct and all identifiable staining was regarded as positive. Within the lesion cyclin positive cells were not diffusely distributed throughout the lesion but appeared as regular foci in individual lesions. When a comparison was made with lymph node germinal centres (Fig. 3), KS lesions showed a lower number of proliferating cells and a weaker reaction product. There was no clear correlation between level of proliferation in individual lesions and "clinical aggressiveness" or anatomical involvement. (Table 1). However, there seems to be a higher mean level of proliferation in HIV positive compared with HIV negative cases (Table 2), but the number examined is too small for statistical evaluation.

DNA measurements

The DNA distribution patterns of individual cases are shown in Fig. 4. In some AKS cases more than 70% of the cells contained DNA values above the 2.5C exceeding rate (Fig. 5a), but no significant percentage of cells with DNA values greater than 5C level were found (Fig. 5b). Both EKS and AKS had a euploid DNA pattern.

DISCUSSION

Kaposi's sarcoma associated with AIDS (AKS) appears clinically more aggressive than endemic (EKS) and classical (CKS), because of its ofen rapid anatomical dissemination suggesting a biologically more malignant KS form. Prognosis in malignant tumours is usually evaluated by clinical staging and histological grading. However, in some tumours morphological criteria are of relatively limited value in assessing the degree of malignancy in terms of prognosis [26]. In KS although grading is feasible, staging is not possible because of its apparent multicentric development. The more recent use of other objective parameters such as DNA ploidy [16, 17], and cell proliferation [18, 19] measurements have improved the characterisation of the biological nature of tumours and the assessment of prognosis. In this study we have used quantitative DNA morphometry and cyclin/(PCNA) immunostaining to compare ploidy and proliferative activity of African AKS and EKS. Our observations indicate that both forms of African KS have an euploid spindle cell population. This extends to African cases similar ploidy findings in AIDS-associated and classical KS from the U.S.

Table 2. Spindle cell proliferation in Kaposi's sarcoma

Diagnosis	Age (mean)	Proliferation* (mean %)
Endemic (HIV−)(n = 7)	56	9.2 (SD 4.95)
Epidemic (HIV+)(n = 10)	29	11.4 (SD 5.88)

*Determined by immunohistochemistry with anticyclin (PCNA, 19F4, NCL-PCNA-10).

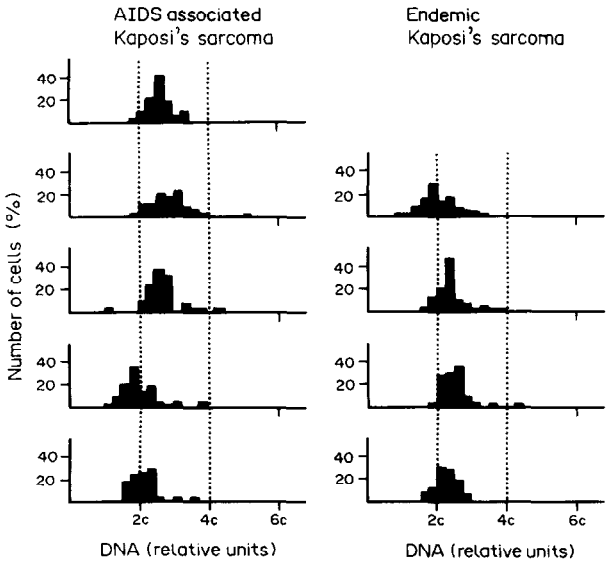

Fig. 4. DNA ploidy distribution patterns of African endemic Kaposi's sarcoma (EKS) and AIDS-associated Kaposi's sarcoma (AKS).

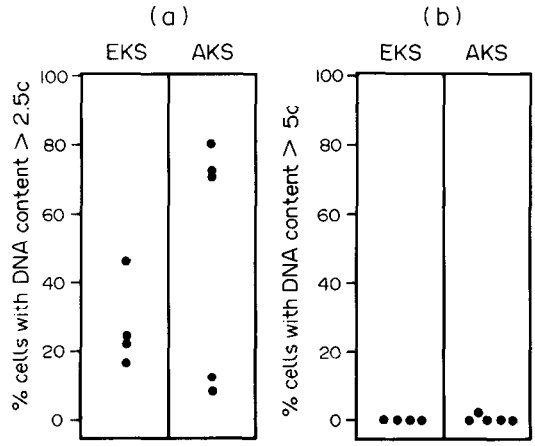

Fig. 5. (a) Percentage of cells with DNA content content greater than 2.5C exceeding rate. (b) Percentage of cells with DNA content greater than 5C exceeding rate.

(NY) [27]. In addition, our study shows that both forms of African KS also have comparable proliferation rates as shown by immunostaining for the S-phase associated nuclear antigen PCNA/cyclin although there seems to be a higher mean level of proliferation in HIV positive compared with HIV negative cases. This is in agreement with the observations that the so called "atypical" KS in Africa is more aggressive (J. Luande, and ref. 28). Th examined number of cases are however, too few for statistical evaluation. This suggests that the more aggressive course of AKS may be related to the immunosuppression and/or other co-factors in these patients. The similarity of AKS and EKS is not only apparent from their common histopathology, cell ploidy and proliferation rates but also from their immunohistochemical features (unpublished data). Although lack of aneuploidy does not necessarily exclude malignant clonal tumour growth [16], these findings strongly support previous suggestions that KS lesions are primarily manifestations of reactive nature [1, 6, 10, 11]. Furthermore, other features of KS are not characteristic of malignant tumours. Thus Safai *et al.* [29], emphasised that KS appears as multiple lesions, rather than a single lesion with secondary metastasis. Likewise consistent chromosomal abnormalities have not been found in KS [30]. Furthermore, KS lesions have been seen to regress spontaneously [10] and in general KS has a low mortality despite its extensive and disseminated growth [2–5]. Noteworthy is the fact that KS is exclusively associated to human species which could favour the suggestion of an unknown human infectious agent [6]. Also contrary to the behaviour of malignant sarcoma, efforts to transplant KS cells have not resulted in malignant growth in nude mice [13]. Recently [12–15], it was shown that AIDS-KS derived cells grown *in vitro* do not display growth characteristics of tumour cells, were diploid and had a normal karyotype. In a recent study (unpublished data), we have demonstrated that a relatively large population of spindle cells have the immune phenotype of myofibroblasts.

Taken together available data seem to indicate that the KS represent a category of reactive lesion which can develop certain degree of autonomous growth. This is probably dependent on the effect of growth factor loops [16], directly or indirectly established by co-factors (infectious agent), particularly in various immunodeficiency states.

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Growth Stimulation of a Human Colorectal Carcinoma Cell Line by Interleukin-1 and -6 and Antagonistic Effects of Transforming Growth Factor β_1

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We analysed the effect of interleukin-1 (IL-1), IL-6 and transforming growth factor β_1 ($TGF\beta_1$) on the growth of a panel of eight colorectal carcinoma cell lines. IL-1 stimulated growth of two lines (LS411N and LS1034) up to20-fold and IL-6 enhanced proliferation of LS1034 more than 5-fold. Both cytokines also augmented colony-formation of LS1034 in methylcellulose. Under both growth conditions IL-1 was the most potent stimulator. However, the addition of IL-6 to IL-1 synergistically enhanced proliferation of LS1034 in monolayer culture and additively augmented the number of colonies formed in methylcellulose. Furthermore, $TGF\beta_1$ strongly reduced the growth rate of LS1034. Low amounts of $TGF\beta_1$ markedly inhibited the response of LS1034 to IL-1 and totally abrogated proliferation induced by IL-6. We conclude that different cytokines can provide distinct signals for the regulation of growth of colorectal carcinoma cells.
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INTRODUCTION

THE TERM CYTOKINES covers a number of soluble mediators that deliver signals from one cell to another. Within this group, the interleukins (IL) represent an important family. The spectrum of target cells affected by a single interleukin is generally rather broad. This is particularly true for IL-1 and IL-6, both molecules exerting activities on a large variety of different cell types.

Peripheral blood monocytes were originally described as the producer cells of IL-1. Evidence has accumulated, however, that numerous other cell types are also capable of secreting IL-1[1]. The effects of IL-1 on tumour cell growth are controversial. For some tumours IL-1 is cytotoxic [2] or cytostatic [3]. In contrast, IL-1 enhances the proliferation of several tumour cell lines [4–6] including one of colorectal origin [7]. The activities of IL-6, originally described as a B cell differentiation factor are also pleiotropic. IL-6 is produced by various tumours and