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Distribution of Immunocompetent Cells in Oral Kaposi's Sarcoma (AIDS)

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Fifteen biopsy specimens of oral AIDS-associated Kaposi's sarcoma (KS), 19 biopsy specimens of uninvolved oral mucosa of HIV-seropositive patients (HIV^+) and 22 biopsy specimens of oral mucosa of HIV-seronegative persons (HIV^-) were analysed for the distribution of $CD4^+$ and $CD8^+$ lymphocytes and $HLA\text{-}DR^+$ cells. The results were statistically evaluated. According to their clinical appearance KS were classified as flat lesions ($n = 10$) or exophytic tumours ($n = 5$). KS lesions of both clinical groups as well as uninvolved mucosa of HIV^+ patients revealed infiltration with $CD4^+$ cells. In flat, patch-like KS there was a marked increase of $CD8^+$ cells compared to HIV^- mucosa, while their numbers decreased in later tumour stages. In both, flat and exophytic KS the number of $HLA\text{-}DR^+$ cells was significantly higher than in uninvolved mucosa of HIV^+ and HIV^- persons. These findings may reflect the local influence of KS growth factors on the inflammatory reaction in the setting of systemic immunosuppression.
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INTRODUCTION

KAPOSI'S SARCOMAS (KS) associated with AIDS frequently occur intraorally, often located in the palatal mucosa or gingival margin [1, 2]. Based on the characteristic features the clinical diagnosis is easily established in most cases. Early KS appear as painless, red to blue macules. In later tumour stages the lesions rapidly grow to form submucosal nodes, which may ulcerate or become superinfected. Occasionally, the tumour may also lack the typical pigmented surface, thus clinically resembling other neoplasms, particularly malignant lymphomas [3] or may occur intraosseously [4].

The clinical behaviour of KS in AIDS patients is unpredictable: the rate of progression varies considerably, with few patients having indolent stagnant disease, while most of the patients develop a fulminant course with early dissemination and multiple organ involvement. The median survival of AIDS patients with KS has been reported as 18–24 months [5], with a 5-year-survival proportion of 8% [6].

Up to now, no specific initial presentation or tumour stage has been predictive of the aggressiveness and subsequent clinical course [7]. The fact that KS lesions, seen in association with the use of immunosuppressive drugs without underlying HIV infection, respond favourably to a discontinuation of the medication suggests that the impaired immune status may determine the clinical presentation. In contrast, patients with only few slow-growing lesions seem to represent a subgroup of patients with a much better prognosis. These patients have normal or moderately impaired immune status, implying that severe immune impairment may not be a prerequisite for the development of KS lesions [8].

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Therefore, it has been speculated whether KS is a true neoplasia or a hyperplasia [9]. Histologically, KS lesions may simulate a number of histopathological conditions, i.e. granulation tissue, haemangiomas, haemangiosarcomas, or fibrosarcomas. The most characteristic feature of KS is spindle-shaped cells with elongated nuclei [10]. They are abundant in the tumour, reveal a low mitosis rate, and are euploid [11]. Many authors believe that these so-called spindle cells are derived from endothelial cells of capillary or lymphatic origin, although staining for endothelial cell markers is inconsistent [11].

Different aetiological factors have been postulated for this tumour. The aetiological relationship of HIV [12, 13], of cytomegalovirus (CMV) [14] and of human papilloma virus (HPV) [15, 16] to KS has been discussed. However, cultivated KS cells are negative for HIV-1 and many other viruses tested. Although ultrastructural evidence of HIV particles has been shown, HIV nucleic acid sequences have not been detected *in situ* [17]. Nevertheless, HIV-1 tat protein can stimulate the proliferation of KS cells *in vitro* [11], although this may happen indirectly by induction of cytokines. Generally, the evolution of KS has been linked with certain growth factors [18]. Apart from specific cytokine receptors KS cells express several growth factors themselves, which promote their own growth as well as that of surrounding cells [19, 20]. They also release factors with chemotactic and chemoinvasive activities [21]; especially granulocyte-macrophage-colony-stimulating factor (GM-CSF) may be responsible for the infiltration of KS with inflammatory cells. On the other hand, T-cells could play a special role in the development and progression of KS, since some growth factors released by activated lymphocytes are able to promote the proliferation of KS cells *in vitro*.

To elucidate further the role of local inflammatory cells in the progression and stage of oral KS, we investigated the expression of CD4, CD8 and HLA-DR antigens in specimens of this tumour and of uninvolved oral mucosa.

MATERIALS AND METHODS

Biopsy specimens were taken from oral KS in 15 HIV^+ patients (hard palate: $n=10$; alveolar mucosa: $n=5$), from clinically uninvolved oral mucosa in 19 HIV^+ patients (gingiva: $n=12$; tongue: $n=7$) and in 22 HIV^- persons (gingiva: $n=15$, tongue: $n=7$).

Specimens of KS were further divided into two different groups according to the clinical stage of flat or exophytic lesion. Flat lesions were defined as multiple red to purple, vascular appearing macules ($n=5$) and exophytic KS as tumours in advanced nodular stages ($n=10$).

Each specimen was bisected: one part was fixed in 10% neutral buffered formalin for routine histopathological evaluation (staining with haematoxylin and eosin), the other part was snap frozen in liquid nitrogen and stored at $-75°C$ for immunohistological examination.

For immunohistochemical studies, monoclonal antibodies were applied according to the APAAP technique [22] against CD4 (Coulter immunology), CD8 (Coulter Immunology) and HLA-DR (Dako).

The slides were microscopically examined using a Leitz Orthoplan microscope with a primary magnification of 250. Cells positive for CD4, CD8 and HLA-DR antigens were counted within random fields. In each specimen four different fields were evaluated. The mean value and standard error of the mean (S.E.) of the number of positive cells/slide were determined. To decide whether there was a significant difference between the results gained from specimens in the different groups the values were examined using an analysis of variance and a subsequent multiple test according to Wilcoxon.

RESULTS

Flat lesions of KS consisted primarily of small aggregates of endothelium-lined vessels filled with erythrocytes, and thin bundles of spindle-shaped cells. All flat lesions contained extravasated erythrocytes and infiltrates of inflammatory cells, mainly consisting of lymphocytes.

In exophytic lesions dense, interlacing fascicles of spindle-shaped cells were observed. Well-formed vascular spaces were less numerous than in flat KS, mainly occurring at the margin of the lesion (Fig. 1).

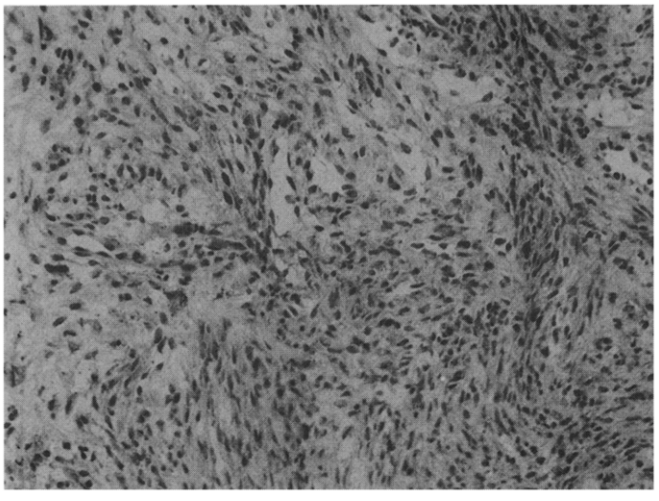

Fig. 1. Late stage KS, clinically classified as exophytic lesion, with dense fascicles of spindle-shaped cells (haematoxylin and eosin × 120).

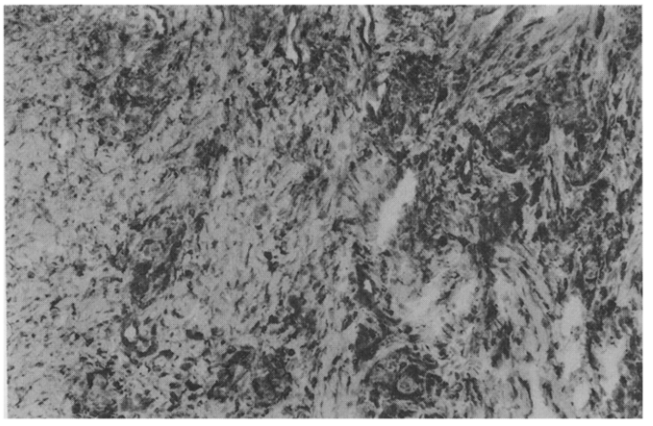

Fig. 2. CD8 positive cells in early stage of KS (APAAP × 160).

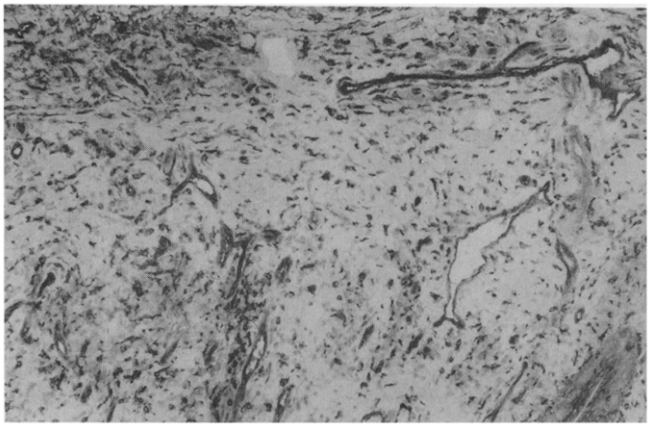

Fig. 3. Expression of HLA DR on inflammatory cells, endothelial cells and some spindle-shaped tumour cells in early stage of KS (APAAP × 160).

In flat lesions $CD4^+$ and $CD8^+$ (Fig. 2) mononuclear cells were mainly associated with vessel-like spaces. $HLA\text{-}DR^+$ cells occurred in clusters of positive cells or were diffusely scattered around blood vessels (Fig. 3).

In exophytic lesions $CD4^+$ and $CD8^+$ inflammatory cells were found mainly around vascular spaces. Mononuclear and some spindle-shaped cells surrounding vessel-like slits as well as endothelial cells stained positive for this HLA-DR (Fig. 4).

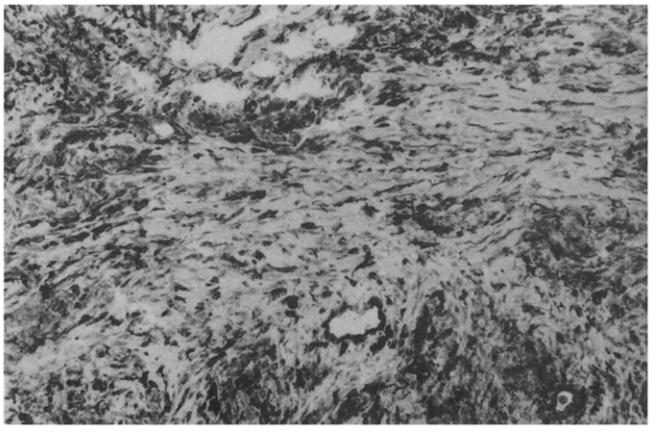

Fig. 4. Positive staining for HLA-DR mainly on inflammatory cells, endothelial cells and spindle-shaped tumour cells in late stage of KS (APAAP × 160).

Although in KS lesions the average number of $CD4^+$ cells was higher than in HIV-mucosa, the difference was not significant statistically (Table. 1).

In contrast, the number of $CD8^+$ cells was significantly increased in flat KS compared with uninvolved oral mucosa of HIV^- persons ($P=0.004$), while similar results were found in patch-like KS and in uninvolved oral mucosa of HIV^+ patients.

In nodular KS, $CD8^+$ cells were increased compared with HIV- mucosa ($P=0.01$), but their number was decreased compared with HIV^+ mucosa ($P=0.0001$) and with patch-like KS ($P=0.01$).

Due to decreased numbers of $CD8^+$ cells within exophytic KS the ratio of CD4/CD8 cells was increased compared with patch-like KS and with HIV^+ mucosa ($P<0.01$). The number of $HLA\text{-}DR^+$ cells was increased within patch-like KS compared to HIV^+ ($P=0.002$) and HIV^- mucosa ($P=0.002$); a similar increase was noted in nodular KS (HIV^- mucosa: $P=0.001$; HIV^+ mucosa: $P=0.001$).

DISCUSSION

Kaposi's sarcoma, the most common malignant tumour associated with AIDS, is a mesenchymal tumour of varying clinical behaviour, histological appearance and of undefined aetiology [8]. The development of this malignancy seems to be favoured by perturbation of the immune system and perhaps antigenic challenge [12, 23]. Whereas definitive identification of a virus in KS has not been sustained, there is increasing evidence of a sexually transmitted aetiological factor [9, 13].

Regarding the histogenesis, molecular events involving a cascade of cytokines and the HIV1 trans-activator gene *tat* seem to mediate the development of a hyperplastic lesion, while secondary genetic changes associated with profound immunosuppression may lead to transformation, tumour proliferation and progression [24].

Thus, initial stages of KS may be considered as a focus of activated cells that may regress or progress, depending on the immune status of the patient and on whether the initial proliferative, probably viral and/or microbial stimulus persists. Through the expression of cytokines with autocrine and paracrine activities [11, 24], the activated cells may permit continued self-renewal, activation and proliferation of surrounding normal cells, such as endothelial cells, fibroblasts, macrophages and lymphocytes; this would be accompanied by neoangiogenesis and by infiltration of inflammatory cells.

Thus, in a vicious circle interactions between cells of the immune system, endothelial cells and fibroblasts may amplify cell activation and cytokine production. In this setting immunodeficiency could play a dual role, since it would both facilitate viral infection and/or reactivation and suppress effector cell functions normally preventing neoplastic cell growth.

This chronological, progressive process may be reflected in the clinical sequence from the early macular lesion to the indurated tumour nodules and the paralleled histopathological changes.

Like the clinical appearance of initial macular lesions, the histopathology of the earliest patch-stage lesions of KS can be difficult to diagnose because of the subtlety of the changes. In the present study, flat patch-like lesions were characterised by a high number of jagged, irregular endothelial-lined vascular spaces and grouped spindle-shaped cells. Characteristically, a few extravasated erythrocytes were found interspersed in the intercellular spaces between spindle cells. A dense mononuclear, inflammatory cell infiltrate composed of plasma cells and lymphocytes was present throughout the tumour, but mainly in areas with extravasated erythrocytes and spindle cells.

Exophytic lesions revealed only very few, thin, endothelial-lined vascular slits surrounded and compressed by dense, interweaving bundles of spindle-shaped cells. Haemosiderin deposition and few extravasated erythrocytes were seen throughout the interstices between the spindle cells. The number of infiltrating mononuclear cells was markedly decreased, while few macrophages were noted within the tumour.

Immunohistologically, the inflammatory cell infiltrates were composed mainly of $CD4^+$ and $CD8^+$ lymphocytes.

T-lymphocytes have important roles in immunoregulation. $CD4^+$ cells include helper and inducer cells, which function as promotors of immunoglobulin production. They are also

Table 1. Distribution of immunocompetent cells

		HIV^-* ($n=22$)	HIV^+† ($n=19$)	KS patch-like† ($n=5$)	KS nodular§ ($n=10$)
CD4	Mean ± S.E.	13.0 ± 1.9	18.0 ± 2.1	25.3 ± 4.0	19.8 ± 1.5
	Range	3.5–38.7	5.5–49.5	6–89	9–60
	Geometr. mean	10.5	14.7	21.2	17.9
	Harm. mean	8.5	12.1	18.0	16.4
CD8	Mean ± S.E.	7.8 ± 0.9	43.5 ± 3.9	35.1 ± 5.8	13.5 ± 1.7
	Range	1.3–18.7	20–96.7	7–90	1–49
	Geometr. mean	6.4	38.9	26.6	10.0
	Harm. mean	4.9	35.2	20.2	6.8
CD4/CD8	Mean ± S.E.	1.8 ± 0.2	0.4 ± 0.03	0.9 ± 0.1	2.4 ± 0.3
	Range	0.9–5.9	0.1–0.9	0.2–2.5	0.4–10
	Geometr. mean	1.6	0.4	0.79	1.8
	Harm. mean	1.5	0.3	0.67	1.3
HLA-DR	Mean ± S.E.	10.9 ± 1.3	6.3 ± 0.5	68.2 ± 7.9	45.7 ± 3.8
	Range	4.2–27.3	2–13.7	3–123	3–96
	Geometr. mean	9.4	5.6	51.2	37.3
	Harm. mean	8.1	4.9	25.2	24.5

*Oral mucosa of HIV-seronegative controll persons, †clinically uninvolved oral mucosa of HIV-seropositive patients, ‡oral patch-like Kaposi's sarcoma of AIDS-patients, §oral nodular Kaposi's sarcoma of AIDS-patients.

important as inducers of cytotoxic T-lymphocytes and of cells which proliferate in response to soluble antigens. In contrast $CD8^+$ cells include precursors to suppressor and cytotoxic cells.

Apart from influences of the systemic immune status, stimulation and variation of local T-lymphocyte subsets can be mediated by viral or other antigens.

Stimulation and activation of the $CD4^+$ subpopulation, which has been described in hyperkeratosis and mild dysplasia [25] would promote immunoglobulin synthesis and secretion of plasma cells against possible "lesion antigens". While in HIV^+ mucosa as well as in KS lesions occasional clusters of $CD4^+$ cells were noted, there was no significant increase of this cell population compared to HIV^- mucosa.

While in the course of HIV-associated disease the absolute number of circulating CD4 cells is reduced, circulating $CD8^+$ suppressor cells are markedly increased. Suppressor cells and their factors may influence effective anti-tumour immune responses and may be responsible for enhanced tumour growth. Furthermore, activation of suppressor cells rather than of cytotoxic cells could also contribute to local immunosuppression and consequent tissue damage.

In flat KS lesions as well as in HIV^+ mucosa the number of $CD8^+$ cells was markedly increased compared to HIV^- mucosa. Infection and/or reactivation of latent infection may be responsible for this infiltration with $CD8^+$ cells. Especially viral antigens could elicit changes in the mononuclear cell phenotypes and may inappropriately induce stimulation of suppressor cells rather than cytotoxic cells. The growing state of HIV-associated immunodeficiency may further lead to the failure of immunosurveillance to eliminate virus-infected cells. As a result, suppression of local immune defense may allow for viral antigens to spread and undergo malignant transformation. Such an inappropriate immunoregulatory response may be reflected in the diminished local presence of $CD8^+$ cells in KS lesions in the advanced, nodular stage.

Major histocompatibility complex (MHC) antigens are highly polymorphic transmembrane glycoproteins. In healthy tissue, class II antigens are generally expressed constitutively on cells of the immune system, mainly on B-cells, monocytes and leucocytic dendritic cells. However, many other cells may be induced to express class II when stimulated by interferon released by activated T cells.

The physiological role of MHC expression has been subject to debate. Increased MHC class II expression may be associated with enhanced antigen presentation to T-helper cells, while in neoplasia inhibition of lymphocyte activation correlated with HLA-DR class expression of tumour cells [26, 27].

In the present study enhanced expression of HLA-DR antigen was observed in KS of the initial and late stages. Almost all endothelial cells stained positive for HLA-DR, while in late nodular lesions some spindle-shaped cells expressed this antigen also. While HLA-DR expression in early KS lesions may be mediated by γ- interferons released from activated lymphocytes, in late tumour stages autocrine and paracrine growth factors produced by KS cells themselves may contribute to enhanced HLA-DR expression.

It is generally supposed that tumours develop in a multistep process [28]. In a first event normally interdependent systems controlling differentiation and proliferation would be uncoupled, resulting in loss of an orderly progression of differentiation. A second transformation event would result in aberrant production of growth factors, mediating autocrine growth stimulation and/or vascularisation [18, 23].

For the evolution of KS, activation of T cells, and the following expression of specific cytokines such as Oncostatin M may play a crucial part [29]. It has been also demonstrated that chronically activated normal lymphocytes have many features in common with immortalised human T-cells and all of these cells produce factors supporting the growth of KS cells [24, 30]. Therefore, continuous local T-cell infiltration, as it has been noted in the present study within HIV^+ mucosa as well as in initial KS, may be of special relevance. Development of KS would thus depend on the balance and strength of local immune reactions, to control antigen invasion and tissue repair.

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