



## Biology of Kaposi's sarcoma

B. Ensoli<sup>a,\*</sup>, C. Sgadari<sup>a</sup>, G. Barillari<sup>a</sup>, M.C. Sirianni<sup>b</sup>, M. Stürzl<sup>c</sup>, P. Monini<sup>a</sup>

<sup>a</sup>Laboratory of Virology, Istituto Superiore di Sanità, Rome, Italy

<sup>b</sup>Department of Clinical Medicine University of Rome "La Sapienza", Rome, Italy

<sup>c</sup>Institute of Molecular Virology, GSF-National Research Center for Environment and Health, Neuherberg, Germany

Received 26 February 2001; accepted 21 March 2001

### Abstract

Kaposi's sarcoma (KS) is an angioproliferative disease occurring in several different clinical-epidemiological forms that, however, share the same histological traits and are all associated with infection by the human herpesvirus 8 (HHV8). KS initiates in a context of immune dysregulation characterised by CD8+ T cell activation and the production of Th1-type cytokines that induce a generalised activation of endothelial cells leading to adhesion and tissue extravasation of lympho-monocytes, spindle cell formation and angiogenesis. These phenomena are triggered or enhanced by infection with HHV8 that, in turn, is reactivated by the same cytokines. Productively-infected circulating cells are recruited into 'activated' tissue sites where HHV8 finds an optimal environment for establishing a persistent, latent infection of KS spindle cells (KSC). HHV8 dissemination is favoured by virus escape mechanisms and immune dysregulation, and leads to immune responses that are not effective against the virus but, paradoxically, exacerbates the reactive process. Although early KS is a reactive process of polyclonal nature that can regress, in time it can progress in to a true sarcoma. The progression of KS appears to be due to the deregulated expression of oncogenes and oncosuppressor genes, to the long-lasting expression of the HHV8 latency genes and, for AIDS-KS, is promoted by the proliferative and angiogenic effects of the HIV-1 Tat protein. © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** KS; Spindle cells; Inflammatory cytokines; Angiogenic factors; AIDS; HIV-1 Tat; HHV8; Angiogenesis; bcl-2; Immune evasion

### 1. Introduction

Kaposi's sarcoma (KS) is a tumour of vascular origin arising with multiple independent lesions of the skin that evolve from flat maculae or patches (early/patch-stage) to plaques (plaque-stage) and then to nodules that can coalesce (late/nodular-stage).

Different clinical and epidemiological forms of KS are recognised. A indolent form is found in elderly men of Mediterranean or Eastern Europe origin, usually arising at the lower extremities and only rarely involving visceral organs (classic KS, CKS) [1]. A mild form of KS is also found in post-transplant patients treated with cyclosporin (PT-KS), particularly in patients from Italy and Saudi Arabia or in certain ethnic groups of Ashkenazi or Shepardnazi Jewish descent [2,3]. An endemic and aggressive form of KS involving visceral and/or

lymphatic organs occurs in young adults and children of sub-equatorial Africa (African KS, AKS) [4,5]. The most aggressive form of KS is found in HIV-1-infected individuals (acquired immunodeficiency syndrome-associated KS, AIDS-KS), where it is generalised and disseminated localising in skin and visceral organs including the gastro-intestinal tract and lungs [6,7]. Among HIV-infected subjects, AIDS-KS is particularly frequent in homo- or bisexual man, that are the groups at highest risk of KS regardless of their ethnical or geographical provenance [7–9].

Although these forms of KS have a different clinical course, they share the same histopathology that is characterised by neoangiogenesis, oedema, erythrocyte extravasation, infiltration of lymphomononuclear cells and growth of spindle-shaped cells (KS spindle cells, KSC). In addition, all forms of KS are associated with infection by the human herpesvirus 8 (HHV8), that is found in KSC from all forms of KS as well as in peripheral blood mononuclear cells (PBMC) from high-risk individuals and patients with KS. These features indicate that the development of the different forms of KS is

\* Corresponding author. Tel.: +39-6-4990-3209; fax: +39-6-4990-3002.

E-mail address: ensoli@iss.it (B. Ensoli).

mediated by the same mechanisms and aetiological agents.

Many observations suggest that, at least in the early stages, KS is not a true sarcoma. For example, KS lesions can simultaneously appear at different sites of the body with a symmetrical or dermatome distribution, can regress spontaneously or following therapy with no recognised antitumour activity [10–13] and, in most cases, are polyclonal in nature and do not show evidence of aneuploidy [14,15]. Other observations, however, suggest the possible malignant nature of late-nodular KS. In fact, although KSC are typically diploid, aneuploid cells have been detected in some high-grade lesions and AIDS-KS lesions can have a high rate of microsatellite instability [14,16,17]. In addition, evidence of clonality of KSC has been recently detected in some advanced lesions from women infected with HIV [18,19].

KS onset is associated with a disturbance of the immune system leading to activation of CD8<sup>+</sup> T cells and increased expression T helper type 1 (Th1)-type cytokines with a high level production of  $\gamma$ -interferon ( $\gamma$ IFN). KS itself starts as a granulation-like tissue rich in inflammatory cells consisting of lymphocytes and monocyte/macrophages. As discussed below, these infiltrating cells, as well as PBMC from patients with KS or at risk of KS, produce the same inflammatory cytokines (IC) including  $\gamma$ IFN, tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-2, IL-6, and others [20,21]. These IC induce the recruitment of circulating cells into tissues through the induction of adhesion and chemotactic molecules, induce the production of angiogenic factors that mediate angiogenesis and oedema, and activate endothelial cells (EC) to acquire the phenotype of KSC [22–27]. Altogether these observations support the concept that early stage KS is a reactive inflammatory-angiogenic process. In this context, recent data suggest that these phenomena may be triggered or enhanced by infection with HHV8 that, in turn, is reactivated by the same IC increased in KS individuals with or at risk of KS [20,21,28]. In fact, in at-risk individuals virus reactivation by IC and lack of immunological control of HHV8 infection may lead to virus spread and dissemination. As indicated by recent studies, HHV8 induces immune responses that, however, are not effective against the virus and, paradoxically, exacerbate the reactive process.

Several studies indicate that the progression of KS into a real tumour is associated with the deregulated expression of oncogenes and oncosuppressor genes, and to the long-lasting expression of HHV8 latency genes in KSC. As discussed below, due to a molecular mimicry of extracellular matrix proteins (ECM), the Tat protein of HIV-1 may act as a progression factor in AIDS-KS by promoting KSC proliferation, migration and invasion [29].

## 2. Histopathology of KS

Lesions from all forms of KS share the same histopathological features such as neoangiogenesis, oedema, infiltration of lymphomononuclear cells, presence of activated proliferating EC forming abnormal blood vessels (slit-like vessels), extravasation of red blood cells, and growth of KSC, that are considered to be the tumour element of KS. As discussed below, spindle-shaped cells are also cultured from the blood of patients with KS or at risk for KS and, like KSC present in lesions, are latently infected by HHV8 which expresses in these cells only a limited sub-set of viral genes.

### 2.1. Histology of KS lesions and origin of KS spindle cells

The first histological change of KS is the appearance of an inflammatory cell infiltrate that precedes the spindle cell formation [20,21,30]. Immunohistochemical analysis of KS lesions shows the presence of T cells, particularly CD8<sup>+</sup> T cells, monocyte-macrophages, and dendritic cells (FXIIIa<sup>+</sup>) (Fig. 1), whereas B cells are few or absent [20,21,31–36]. Like PBMC from patients with KS or at risk of KS, these cells produce Th1-type cytokines including  $\gamma$ IFN, TNF $\alpha$ , IL-1 $\beta$ , IL-6 and others (Table 1) [20,21,37,38]. As discussed below, these cytokines activate EC to acquire the KS cell phenotype and to produce angiogenic factors that mediate angiogenesis, oedema and KS cell growth and locomotion (Table 1) [20,23,25,27,39–42]. In early lesions, KSC are few and intermingled with stromal cells; however, in time KSC fill the stroma between vascular spaces and KS lesions acquire a more monomorphic aspect resembling a fibrosarcoma.

Most studies indicate that lesional KSC consist of a heterogeneous cell population dominated by activated vascular and lymphatic EC (E-KSC) mixed with cells of macrophagic origin (M-KSC) (Fig. 2) [20,21,23,31,33,43–51]. Furthermore, similar to the so-called endothelial-macrophages of the lymph nodes, some KSC co-express markers of monocyte-macrophages and the vascular-endothelial-cadherin [35,36], an adhesion molecule that is expressed by vascular endothelial cells. Spindle-shaped cells with the endothelial-macrophages phenotype can also be cultured from PBMC of patients with KS or at risk for KS, particularly in the presence of the same IC that are produced by PBMC from KS patients and by inflammatory cells infiltrating KS lesions (Fig. 2) [28,36,52,53]. These data suggest that KSC and endothelial macrophages are related cell types and that circulating KSC may be the cell progenitors of lesional KSC. The recruitment into tissues of KSC progenitors may lead to the appearance of multiple lesions at independent sites.

Table 1  
Expression and role of the major host and viral cytokines, angiogenic and chemotactic factors in KS

Factor	Expression <sup>a</sup> <i>in vivo</i>	Expression <sup>a</sup> <i>in vitro</i>	Possible role in KS pathogenesis (additional references not cited in text are included)
IL-1 $\alpha$ , $\beta$	+	+	Activation and induction of a spindle morphology in EC, induction of E-KSC proliferation (mediated by bFGF); cell recruitment into tissues and cell differentiation; increased in patients with KS and individuals at risk of KS (Hober and colleagues <i>Clin Exp Immunol</i> 1989, <b>78</b> , 329–333, 1989; Lepe-Zanuga and colleagues <i>J Clin Microbiol</i> 1987, <b>5</b> , 1695–1700); upregulation of HHV8 gene expression (in PEL cell lines) (Yu and colleagues <i>AIDS</i> 1999, <b>13</b> , 2178–2180); see also references in text.
TNF $\alpha$ , $\beta$	+	–	Induction of E-KSC proliferation; activation and induction of a spindle morphology in EC; cell recruitment and differentiation; increased in patients with KS and individuals at risk of KS (Oxholm and colleagues <i>Acta Pathol Mic Sc</i> 1989, <b>97</b> , 533–538; Fagiolo and colleagues <i>Eur J Immunol</i> 1993, <b>23</b> , 2375–2378; Hober and colleagues <i>Clin Exp Immunol</i> 1989, <b>78</b> , 329–333; Lahdevirta and colleagues <i>Am J Med</i> 1988, <b>85</b> , 289–291; Rizzardini and colleagues <i>AIDS</i> 1998, <b>12</b> , 2387–2396; Vyakarnam and colleagues <i>Biochem Biophys Res Commun</i> 1992, <b>183</b> , 1167–1174); KS progression upon systemic administration (Aboulafia and colleagues <i>J Acquir Immune Defic Syndr Hum Retrovirol</i> 1989, <b>2</b> , 54–58); see also references in text.
$\gamma$ IFN	+	–	Activation and induction of a spindle morphology in EC; cell recruitment and differentiation, induction of Tat-responsiveness; <i>in vitro</i> growth of lesional M-KSC; increased in patients with KS and individuals at risk of KS (Fan and colleagues <i>J Immunol</i> 1993, <b>151</b> , 5031–5040; Rizzardini and colleagues <i>AIDS</i> 1998, <b>12</b> , 2387–2396; Vyakarnam and colleagues <i>Biochem Biophys Res Commun</i> 1992, <b>183</b> , 1167–1174); KS progression after systemic administration (Krigel and colleagues <i>J Biol Response Mod</i> 1989, <b>8</b> , 359–365); reactivation of HHV8 infection (in PBMC and PEL cell lines) (Blackbourn and colleagues <i>AIDS</i> 2000, <b>14</b> , 98–99; Chang and colleagues <i>Virology</i> 2000, <b>266</b> , 17–25; Mercader and colleagues <i>Am J Pathol</i> 2000, <b>156</b> , 1961–1971); see also references in text.
bFGF	+	+	Potent inducer of E-KSC proliferation and angiogenesis; induction of MMPs expression; synergy with HIV-1 Tat protein and VEGF in induction of angiogenesis and oedema; increased in sera from patients with KS or at risk of KS (Ascherl and colleagues. <i>AIDS Res Hum Retrov</i> 2001, in press); expressed at high levels in KS lesions (Xerri and colleagues <i>Am J Pathol</i> 1991, <b>138</b> , 9–15); expression and release induced by IC in EC and E-KSC (Faris and colleagues <i>AIDS</i> <b>12</b> , 19–27); see also references in text.
VEGF	+	+	Synergy with bFGF in inducing angiogenesis and oedema; induced or upregulated by IC in EC or KSC; highly expressed in KS lesions and by cultured E-KSC; cognate receptor expressed in KS lesions (Brown and colleagues <i>Am J Pathol</i> 1996, <b>148</b> , 1065–1074); increased in sera from patients at risk of KS (Ascherl and colleagues <i>Blood</i> 1999, <b>93</b> , 4232–4241); induced in T cells by IC or HIV infection (Ascherl and colleagues <i>Blood</i> 1999, <b>93</b> , 4232–4241) transformed cell lines from KS lesions, but not E-KSC, proliferate in response to VEGF A or C (Masood and colleagues <i>Proc Natl Acad Sci USA</i> 1997, <b>94</b> , 979–984; Marchio and colleagues <i>J Biol Chem</i> 1999, <b>274</b> , 27617–27622; Weindel and colleagues <i>Biochem Biophys Res Commun</i> 1992, <b>183</b> , 1167–1174); see also references in text.
SF/HGF	+	+	Proliferation of E-KSC, cognate receptor expressed in KS lesions (Maier and colleagues <i>Int J Cancer</i> 1996, <b>65</b> , 168–172; Naidu and colleagues <i>Proc Natl Acad Sci USA</i> 1994, <b>91</b> , 5281–5285); reactivation of HHV8 infection (in PEL cell lines) (Mercader and colleagues <i>Am J Pathol</i> <b>156</b> , 2000, 1961–1971); see also references in text.
PDGF-B	+	n.a.	Angiogenic effects, growth effects for KSC; cognate receptor expressed in KS lesions and cultured KSC (Roth and colleagues <i>Oncogene</i> 1989, <b>4</b> , 483–487; Werner and colleagues <i>Exp Cell Res</i> 1990, <b>187</b> , 98–103); see also references in text.
MCP-1	+	+	Recruitment of circulating cells; expression induced by IC (Mantovani and colleagues <i>Immunol Today</i> 1997, <b>18</b> , 231–240) see also references in text.
IL-8	+	+	Recruitment of circulating cells, angiogenic effects; expression induced by IC; see references in text.
RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$ , IP-10, Mig	+	n.a.	Recruitment of circulating cells, modulation of KS angiogenesis (?); expression induced by IC (Sgadari and colleagues <i>Blood</i> 1996, <b>87</b> , 3877–3882; Liao and colleagues <i>J Exp Med</i> 1995, <b>182</b> , 1301–1314; Sgadari and colleagues <i>Blood</i> 1997, <b>89</b> , 2635–2643); see also references in text.
v-IL6	$\pm$	–	Angiogenic effects (mediated by VEGF), but absent or very low level expression in KS lesions (Aoki and colleagues <i>Blood</i> 1999, <b>93</b> , 4034–4043; Staskus and colleagues <i>J Virol</i> 1999, <b>73</b> , 4181–4187; Cannon and colleagues <i>J Infect Dis</i> 1999, <b>180</b> , 824–828); see also references in text.
v-MIPs	+	–	Recruitment of cells into tissues; angiogenic in the chorioallantoic membrane assay; role in KS unclear due to: (i) high level expression of host angiogenic factors; (ii) v-MIPs are chemotactic for Th2 but not Th1 cells or are potent inhibitors of monocyte chemotaxis (Boshoff and colleagues <i>Science</i> 1997, <b>278</b> , 290–294; Kledal and colleagues <i>Science</i> 1997, <b>277</b> , 1656–1659; Stürzl and colleagues <i>Aids</i> 1998, <b>12</b> , 1105–1106; Endres and colleagues <i>J Exp Med</i> 1999, <b>189</b> , 1993–1998; Sine and colleagues <i>Blood</i> 2000, <b>95</b> , 1151–1157); see also references in text.

Table 1 (continued)

Factor	Expression <sup>a</sup> <i>in vivo</i>	Expression <sup>a</sup> <i>in vitro</i>	Possible role in KS pathogenesis (additional references not cited in text are included)
v-GPCR	±	–	Angiogenic effects (mediated by VEGF); development of KS-like lesions in transgenic mice (due to VEGF production); upregulated by IL-8; role in KS unclear due to: (i) transient expression due to HHV8 lytic infection; (ii) downmodulated by IP-10 and v-MIP II; (iii) high level expression of VEGF by KSC (that are negative for v-GPCR expression) (Bais and colleagues <i>Nature</i> 1998, <b>391</b> , 86–89; Yang and colleagues <i>J Exp Med</i> 2000, <b>191</b> , 445–454; Kirshner and colleagues <i>J Virol</i> 1999, <b>73</b> , 6006–6014; Geras Raaka and colleagues <i>Biochem Biophys Res Commun</i> 1998, <b>253</b> , 725–727; Geras Raaka and colleagues <i>J Exp Med</i> 1998, <b>188</b> , 405–408; Sodhi and colleagues <i>Cancer Res</i> 2000, <b>60</b> , 4873–4880) see also references in text.

E-KSC, endothelial KSC; EC, endothelial cells IL, interleukin; TNF, tumour necrosis factor; IFN, interferon; v-IL6: viral interleukin 6; v-MIP, viral macrophage inflammatory protein; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; SF/HGF, scatter factor/hepatocyte growth factor; PDGF, platelet-derived growth factor; v-GPCR, viral G protein coupled receptor; IP-10, interferon-gamma-inducible protein-10; Th1/Th2; T helper type 1 or 2; KS Kaposi's sarcoma; MCP-1, monocyte chemotactic protein-1; RANTES, regulated upon activation, normal T cell expressed and secreted; Mig, monokine induced by gamma-IFN; n.a., not available.

## 2.2. HHV8 infection in KS lesions

HHV8 infection of EC and KSC is a specific trait of KS lesions. In fact, EC present in KS lesions, lesional E-KSC and M-KSC, and the circulating KSC from a patient's PBMC are all infected by HHV8 [28,43,53,55–60]. However, most studies indicate that only a small fraction of the cells is infected in early-patch lesions, whereas both viral load and gene expression increase as lesions progress to more advanced stages and are maximal in late-nodular lesions (Table 2) [43,53–60]. EC and KSC present in early and advanced lesions express HHV8 latency genes including a cyclin D homologue (v-cyc D), a homologue of FLICE inhibitory proteins (FLIPs) (v-FLIP), a family of proteins (kaposins) with as yet unknown functions, and the HHV8 latency-associated nuclear antigen (LANA) (Table 2) [43,54,56–61]. In contrast, productive (lytic) infection is always low and confined to a very small fraction of cells including monocytes and T cells infiltrating KS lesions [54,60,62]. Lytically-infected cells express the whole spectrum of HHV8 genes including viral genes with paracrine effects [63] such as viral IL-6 (v-IL6), several viral homologues of the macrophage inflammatory proteins (v-MIP I, II and III), and a chemokine receptor homologue (v-GPCR) endowed with paracrine actions due to the capability of inducing vascular endothelial growth factor (VEGF) (Table 1). In addition, these cells produce mature viral particles [62] providing a reservoir of virus that may be required for persistent infection of KSC, as suggested by the loss of HHV8 infection upon culture of KSC from lesions [64,65].

## 3. The natural history of KS

KS initiation is the result of the complex interaction of several factors including Th1-type cytokines induced

by infectious agents including HIV and HHV8 or by as yet unknown factors. In time, KS progresses to a true sarcoma, most likely due to the deregulated expression of oncogenes and oncosuppressor genes and to the effects of viral genes endowed with proliferative and anti-apoptotic properties, including HHV8 latency genes and the HIV-1 Tat protein. In addition, immune evasion mechanisms, immune defect or overt immune suppression also play a role in KS initiation or progression by allowing uncontrolled HHV8 replication or tumour growth (see below).

### 3.1. Risk factors for KS development: Th1-type immune activation and HHV8 infection

Recent studies have indicated that both a disturbance of the immune system characterised by immunoactivation accompanied or followed by a more or less pronounced immune defect and infection by HHV8 are two major risk factors for KS development.

CD8 activation leading to the production of Th1-type cytokines is a key factor for KS initiation as indicated by one or more signs of CD8 T cell activation, including soluble CD8, neopterin, increased levels of IC (particularly  $\gamma$ IFN, IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ), or an oligoclonal expansion of CD8+ T cells that are found in patients with AIDS-KS, CKS and AKS, or in individuals at risk of KS such as homo-bisexual men and HIV-1-infected individuals, African subjects and elderly man of Mediterranean origin (Table 1) [20,21,66–71], (data not shown). A key role of Th1-type cytokines in KS initiation is also indicated by studies showing that the administration of  $\gamma$ IFN or TNF $\alpha$  to HIV-1-infected patients leads to KS development or KS progression (Table 1). KS onset is also observed in HIV-1-infected patients during opportunistic infections that are associated with IC production [72]. In addition, despite immunosuppressive therapy, immunoactivation may

also occur in PT-KS where allogeneic stimulation may induce local foci of activated immune cells. It is noteworthy to point out that KS can occur in homo-bisexual men in the absence of HIV-1 infection or overt immunosuppression [73], indicating that immunoactivation and not immune suppression leads to KS initiation.

However, patients that develop KS most often present in a variable degree also a state of immunosuppression including subtle immune defects, as can occur in CKS or AKS [71,74–76], or overt immunosuppression or immunodeficiency, as in PT-KS or AIDS-KS. As discussed below, Th1-type activation, immune defect and immune evasion mechanisms disrupt the normal interaction of HHV8 with the host allowing the spread of HHV8 infection to circulating cells and tissues, or allowing infected KSC to escape immune cytotoxic responses.

A variety of polymerase chain reaction (PCR)-based and serological studies have indicated that HHV8 infection acts as a cofactor in KS development. Evidence for this is based on the following findings: (i) HHV8 infection is more prevalent in countries with a high incidence of KS such as certain areas of Africa, Eastern Europe, Greece and Italy, compared with other

geographical areas where both HHV8 infection and KS incidence are low [1,77–83]; (ii) an increased HHV8 seroprevalence and load are found in population groups at risk for KS including homo/bisexual men and HIV-1-infected individuals [78,80,81,84–86]; (iii) both high antibody titres against HHV8 and PBMC-associated viraemia are predictive of KS development in at-risk individuals [84,87–91].

However, other data indicate that HHV8 requires additional factors to exert its effects in KS development. In fact, in Mediterranean regions where KS is endemic the prevalence of HHV8 infection in the general population is exceedingly high compared with KS incidence [1,83]. Moreover, recent studies have indicated that HHV8 seroprevalence in Africa remained unchanged upon the sharp increase of KS incidence associated with the HIV-1 epidemic [77], pointing to HIV-1 infection as an independent risk factor for the development of KS.

### 3.2. The reactive and tumour stages of KS

Early stage KS, at least, may not be a true sarcoma, but rather a hyperplastic reactive-inflammatory process.

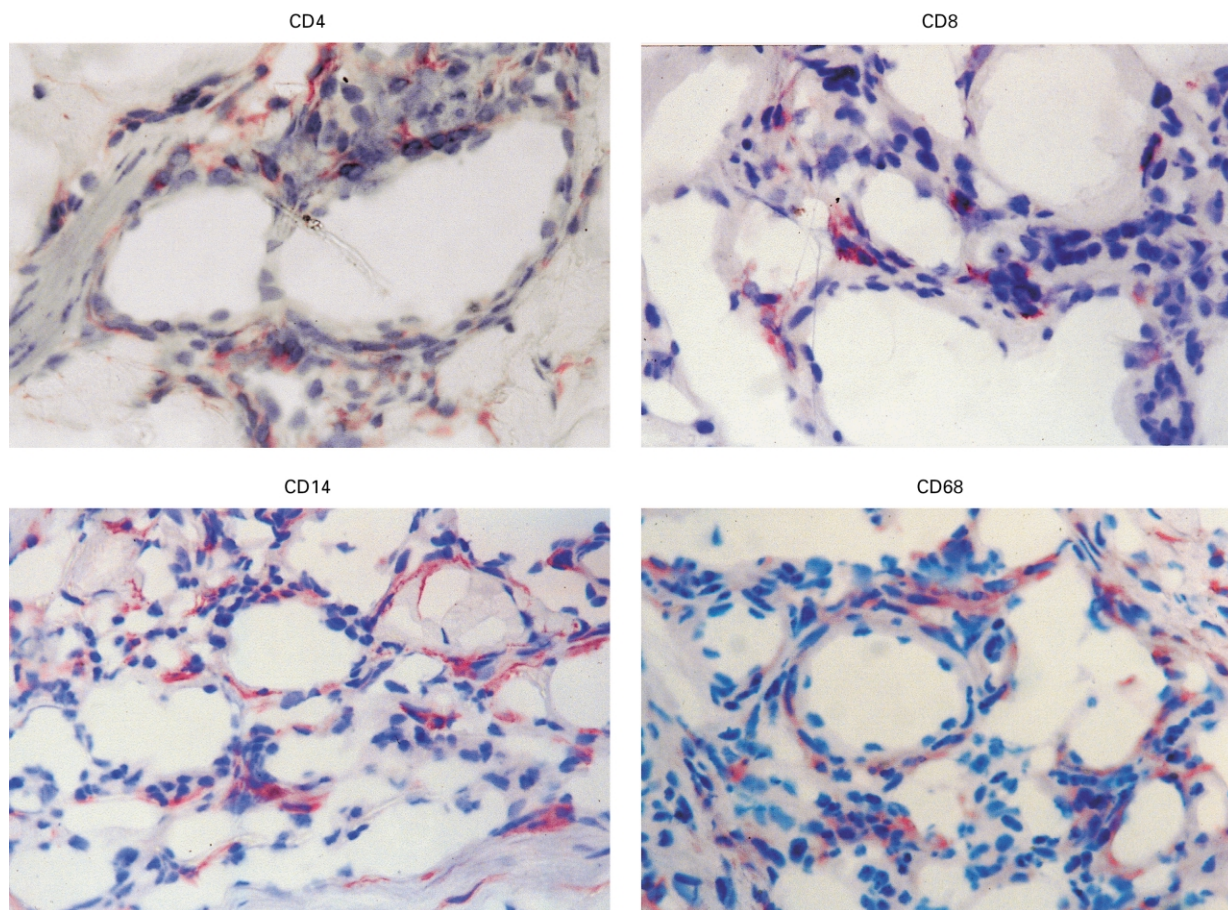


Fig. 1. Detection of inflammatory cells, including CD4, CD8, CD14, or CD68-positive cells, infiltrating a early-stage human KS lesion. CD14 and CD68-positive cells include spindle-shaped cells, often with a sub-endothelial localisation. These cells express Th1-type cytokines including IL-1 $\beta$  and TNF  $\alpha$  and high levels of  $\gamma$ IFN [20,21] (immunohistochemistry, alkaline phosphatase anti-alkaline phosphatase (APAAP), 100 $\times$  magnification).

This is suggested by the simultaneous appearance of multiple symmetrical lesions developing in the absence of metastasis [10], by the disappearance of PT-KS upon withdrawal of the immunosuppressive therapy [92], or by the regression of AIDS-KS that can occur either spontaneously or upon treatment of HIV-infected patients with the highly active antiretroviral therapy (HAART) [10–13]. The reactive, non-neoplastic nature of KS is also indicated by the polyclonal nature of early lesions, as determined by the analysis of the methylation

pattern of the androgen receptor gene [15]. In agreement with these data, HHV8 genomic terminal repeats (TR) also show a polyclonal or oligoclonal pattern in most KS lesions analysed, whereas HHV8 TR have a clonal pattern in primary effusion lymphoma (PEL), a rare type of lymphoma associated with HHV8 infection [93]. These findings suggest that persistent, latent infection of KSC most likely occurs through the continuous exposure of KSC to infectious virus released by productively infected lympho-monocytes present in lesions, as also

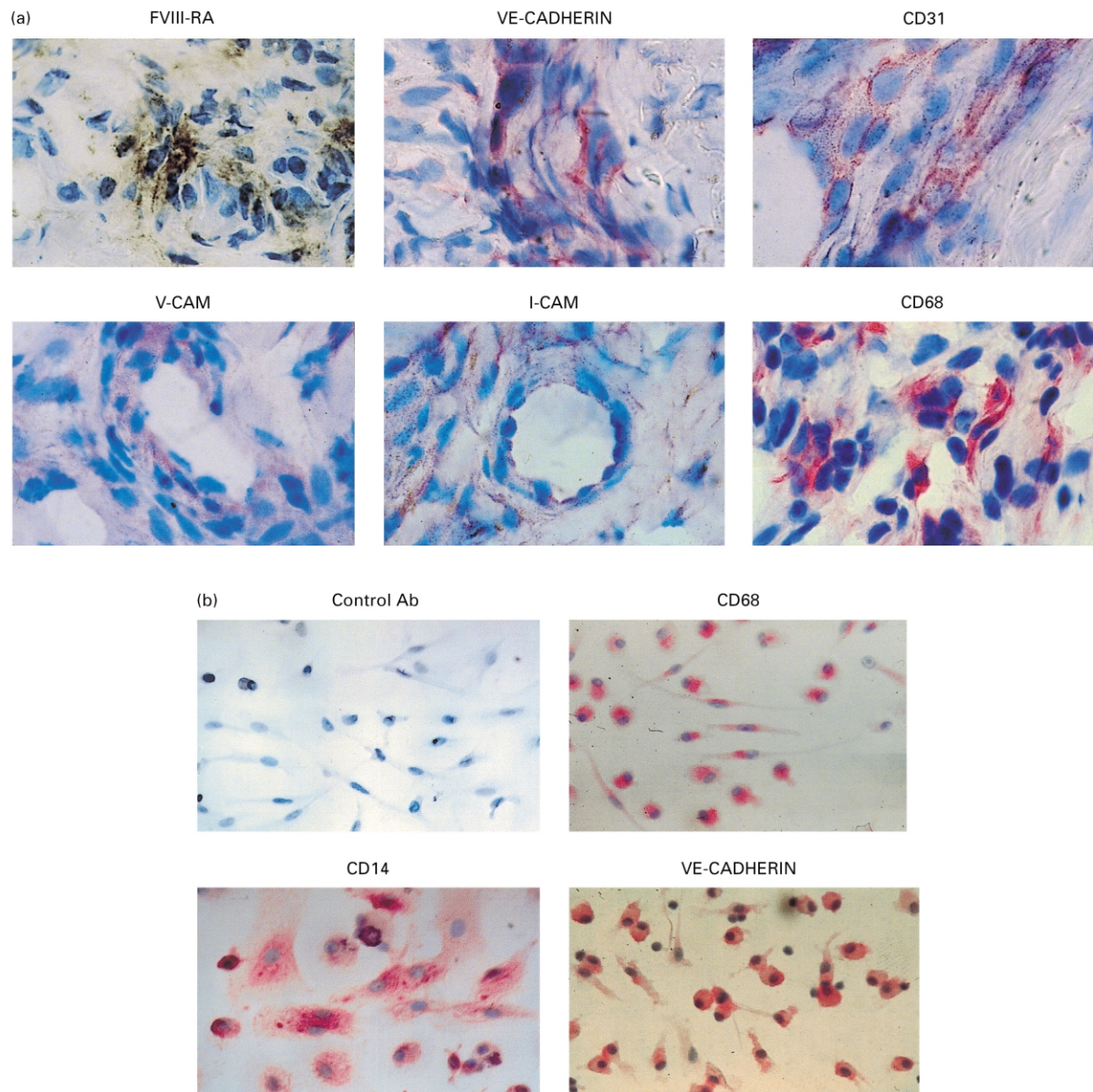


Fig. 2. Detection of KS spindle cells in a human KS lesion and spindle cells obtained upon culture of PBMC from a patient with AIDS-KS. (a) Spindle cells present in the KS lesion stain for markers typical of endothelial cells (EC) including FVIII-related antigen (FVIII-RA) and adhesion molecules expressed by activated EC such as vascular-endothelial (VE)-cadherin, intercellular adhesion molecule (I-CAM) or vascular adhesion molecule (V-CAM). Several spindle cells, in addition, stain for monocytic cell markers such as CD68 (peroxidase anti-peroxidase; or APAAP method, 100 $\times$  magnification). (b) Phenotypic characterisation of adherent cells obtained from PBMC of an AIDS-KS patient cultured for 6 days in the presence of IC including IL-1 $\beta$ , TNF $\alpha$  and  $\gamma$ IFN (modified with permission from Monini and colleagues [28] and The American Society of Hematology). Adherent cells with the typical spindle morphology staining for CD68, CD14 and VE-cadherin are shown (40 $\times$  magnification).

Table 2  
Host and viral factors potentially involved in KS progression

Factor	Expression in early lesions	Expression in nodular lesions	Possible role in KS pathogenesis (additional references not cited in text are included)
Bcl-2	±	++	KSC and EC survival; KSC proliferation; transformation of reactive KS (?); Bcl-2 expression induced by vFGF and Tat; down-regulated by paclitaxel, a drug inducing KS regression (Dada and colleagues <i>Histopathology</i> 1996, <b>29</b> , 159–163; Pillay and colleagues <i>Pathol Oncol Res</i> 1999, <b>5</b> , 17–20) see also references in text.
p53	±	++, mutated	KSC proliferation and survival (?) (Scincariello and colleagues <i>J Virol</i> 1994, <b>203</b> , 153–157; Dada and colleagues <i>Histopathology</i> 1996, <b>29</b> , 159–163; Hodak and colleagues <i>Am J Dermatopathol</i> 1999, <b>21</b> , 138–145; Noel and colleagues <i>Arch Dermatol Res</i> 1997, <b>289</b> , 660–661; Pillay and colleagues <i>Pathol Oncol Res</i> 1999, <b>5</b> , 17–20).
myc	±	++	Induced by PDGF-B; KSC proliferation inhibited by c-myc antisense oligonucleotides (Spandidos and colleagues <i>Anticancer Res</i> 1990, <b>10</b> , 1619–1625; Köster and colleagues <i>Am J Pathol</i> 1996, <b>149</b> , 1871–1885; Kaaya and colleagues <i>Med Oncol</i> 2000, <b>17</b> , 325–332).
ras	+	+, mutated	Transformation of reactive KS (?) (Spandidos and colleagues <i>Anticancer Res</i> 1990, <b>10</b> , 1619–1625; Nicolaidis and colleagues <i>Anticancer Res</i> 1994, <b>14</b> , 921–926).
int-2	+, mutated	+, mutated	Angiogenesis (?) KSC proliferation (?) (Huang and colleagues <i>J Clin Invest</i> 1993, <b>91</b> , 1191–1197; Kiuru-Kuhlefelt and colleagues <i>Mod Pathol</i> 2000, <b>13</b> , 433–437).
v-cyc D	±	++	Phosphorylation of Rb protein (CDK-inhibitors independent); degradation of p27 <sup>Kip</sup> ; activates cyclin A; Bcl-2 inactivation (upon virus productive replication); induction of KSC proliferation (?) (Reed and colleagues <i>Blood</i> 1998, <b>91</b> , 3825–3832; Ellis and colleagues <i>EMBO J</i> <b>18</b> , 1999, 664–653; Godden-Kent and colleagues <i>J Virol</i> 1997, <b>71</b> , 4193–4198; Mann and colleagues <i>EMBO J</i> 1999, <b>18</b> , 654–663; Swanton and colleagues <i>Nature</i> 1997, <b>390</b> , 184–187; Ojala and colleagues <i>Nat Cell Biol</i> 2000, <b>2</b> , 819–825); see also references in text.
v-FLIP	±	++	Survival of KSC (through protection from FAS/TNF-mediated apoptosis and from CTL cytotoxic activity) (Bertin and colleagues <i>Proc Natl Acad Sci USA</i> 1997, <b>94</b> , 1172–1176; Djerbi and colleagues <i>J Exp Med</i> 1999, <b>190</b> , 1025–1032; Thome and colleagues <i>Nature</i> 1997, <b>386</b> , 517–521); see also references in text.
Kaposin A, B, C	±	±	Function unknown; Kaposin A transforms rodent cells (Zhong and colleagues <i>Proc Natl Acad Sci USA</i> 1996, <b>93</b> , 6641–6646; Sadler and colleagues <i>J Virol</i> <b>73</b> , 5722–5730; Muralidhar and colleagues <i>J Virol</i> 1998, <b>72</b> , 4980–4988; Muralidhar and colleagues <i>J Clin Virol</i> 1998, <b>16</b> , 203–213); see also references in text.
LANA	±	++	Maintenance of virus DNA in latently infected cells (Bellestas and colleagues <i>Science</i> 1999, <b>284</b> , 641–644); p53 inactivation (Friborg and colleagues <i>Nature</i> 1999, <b>402</b> , 889–894); interference with the Rb/E2F complex (Radkov and colleagues <i>Nat Med</i> 2000, <b>6</b> , 1121–1127); modulation of host gene expression (Platt and colleagues <i>J Virol</i> 1999, <b>73</b> , 9789–9795; Krithivas and colleagues <i>J Virol</i> 2000, <b>74</b> , 96370–9645; Renne and colleagues <i>J Virol</i> 2000, <b>75</b> , 458–468). See also: Rainbow and colleagues <i>J Virol</i> 1997, <b>71</b> , 5915–5921; Kedes and colleagues <i>J Clin Invest</i> 1997, <b>100</b> , 2606–2610; Katano and colleagues <i>Am J Pathol</i> <b>155</b> , 47–52; and references in text.
Tat	+	+	Proliferation of KSC and IC-activated EC; enhancement of angiogenesis and KSC invasion (in synergism with IC bFGF); upregulation of MMPs; induction of Bcl-2 expression; release of bFGF bound to HSPG; binding to integrin receptors; activation of p125 FAK (Milani and colleagues <i>AIDS</i> 1998, <b>12</b> , 1275–1284; Ganju and colleagues <i>J Virol</i> 1998, <b>72</b> , 6131–6137; B. Ensoli and colleagues, data not shown); binding to the VEGF receptor Flk-1/KDR-1 (Albini and colleagues <i>Nat Med</i> 1996, <b>2</b> , 1371–1375; Ganju and colleagues <i>J Virol</i> <b>72</b> , 1998, 6131–6137); activation of IC expression (Chang and colleagues <i>J Biomed Sci</i> 1995, <b>2</b> , 189–202; Buonaguro and colleagues <i>J Virol</i> 1994, <b>68</b> , 2677–2682; Scala and colleagues <i>J Exp Med</i> 1994, <b>179</b> , 961–971); induction of adhesion molecules in E-KS and EC (Hoffman and colleagues <i>Blood</i> 1993, <b>82</b> , 2774–2780; Kelly and colleagues <i>AIDS</i> 1998, <b>12</b> , 1753–1761; Dhawan and colleagues <i>Blood</i> 1997, <b>90</b> , 1535–1544); see also Albini and colleagues <i>Proc Natl Acad Sci USA</i> 1995, <b>92</b> , 4838–4842, and references in text.
Overt immune deficiency	n.a.	n.a.	Associated with microsatellite instability in KSC (?); see references in text.

KSC, KS spindle cells, EC, endothelial cells; v-FLIP, viral FLIPs, FLICE (FADD (Fas-associated death domain)-like interleukin 1 $\beta$  converting enzyme) inhibitory proteins; v-cyc D, viral cyclin D; LANA, latency-associated nuclear antigen; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; Rb, retinoblastoma; CDK, cyclin-dependent kinase; IC, inflammatory cytokines; HSPG, heparan sulphate proteoglycans; FAK, focal adhesion kinase; FAS/TNF, CD95 tumour necrosis factor; CTL, cytotoxic T lymphocyte; n.a., not applicable.

indicated by the loss of HHV8 DNA from E-KSC upon culture [64,65]. These data supports the concept that KS initiates as a reactive process, and suggests that in early-stage KS HHV8 acts by participating in the reactive process rather than by transforming KSC to a malignant phenotype. Accordingly, most studies have indicated that E-KSC do not have the features of malignant cells [15,48,94,95]. By contrast, these cells have an activated phenotype and after inoculation in nude mice they promote the formation of highly angiogenic KS-like lesions of mouse cell origin that is mediated by the paracrine action of cytokines and angiogenic factors produced by the cells [29,41,96,97]. Indeed, as discussed below, E-KSC appear to be 'trans-differentiated' cells due to the acquisition of phenotypic and functional traits that are not found in normal endothelial cells.

However, aneuploidy, microsatellite instability and clonality of KSC and HHV8 TR have been found in advanced KS [17–19,93] and few transformed KS cell lines have been established from nodular lesions, indicating that late-stage KS can be transformed and monoclonal [98,99]. As discussed below, the long-term expression of the HHV8 latency genes associated with the deregulated expression of oncogenes or oncosuppressor genes, such as *c-myc*, *Bcl-2*, and *TP53*, may play a key role in the transformation of KS into a true sarcoma (Table 2). In addition, the HIV-1 Tat protein may act as a progression factor in AIDS-KS by inducing the growth, migration and invasion of KSC [28,39] and IC-activated EC [23–26,29] (see below). In this context, overt immune deficiency may facilitate KS progression due to the lack of control of tumour growth leading to chromosomal changes, as suggested by the presence of microsatellite instability in AIDS-KS, but not in CKS [17].

#### 4. KS initiation and development

The IC upregulated in immunoactivated individuals at risk of KS play a key role in KS initiation. In fact, experimental studies indicate that they induce several events associated with KS development since (i) they activate EC to express adhesion molecules and induce chemokines that recruit circulating cells into tissues, including HHV8-infected cells; (ii) they induce EC to acquire the phenotypic and functional properties of KSC including the responsiveness to the effects of extracellular HIV-1 Tat protein; and (iii) they induce production of angiogenic factors that mediate EC and KSC proliferation, angiogenesis, oedema and lesion growth.

Moreover, IC provide a major stimulus for HHV8 reactivation in circulating cells leading to virus spread and dissemination in tissues. In turn, HHV8 dissemina-

tion induces immune responses that are not capable of controlling virus infection and, paradoxically, induce or exacerbate the reactive processes initiating KS lesions (see below).

##### 4.1. Effects of Th1-type cytokines on endothelium activation, cell recruitment into tissues, and spindle cell formation

The systemic and local production of IC found in individuals at risk of KS or with KS activate the vascular endothelium and induce the extravasation and tissue recruitment of inflammatory cells. In fact, HIV-1-infected patients show increased endothelial cell permeability and increased serum levels of FVIII-RA, which is released by activated EC, and a generalised activation of the vessel endothelium with expression of adhesion molecules (I-CAM-1, ELAM-1, V-CAM-1); this leads to increased adhesion and extravasation of lymphomonocytes that also produce IC [20–22,100,101]. Due to these processes, the endothelium from individuals at risk of KS is characterised by the presence of 'activated tissue sites', that may be sites prone to KS lesion development [22]. These observations are also supported by the presence in uninvolved skin from KS patients, but not skin from subjects with other dermatological disorders, of tissue foci expressing  $\gamma$ IFN and human leucocyte antigen DR (HLA-DR) [20].

Other observations suggest that IC can trigger a cascade of events leading to KS lesion formation, as indicated by the development of KS-like lesions upon IC inoculation in mice [25]. In fact, the same IC increased in KS and in individuals at risk of KS including IL-1 $\beta$ , TNF $\alpha$  and  $\gamma$ IFN, activate normal EC to acquire the phenotypic and functional features of E-KSC (Table 1). These include the typical spindle cell morphology, the expression of the same markers (downregulation of FVIII-RA, activation of ELAM-1, ICAM-1, VCAM-1, DR,  $\alpha$ 5 $\beta$ 1 and  $\alpha$ v $\beta$ 3 integrin expression), and the angiogenic phenotype [20,23,25,27,39,41,42]. IC, in particular, induce EC to promote the formation of KS-like lesions after inoculation in nude mice, a property shown by E-KSC [20,23,27,29,42,96,97].

As discussed below, IC also induce normal EC to become responsive to the adhesive, mitogenic and invasive effects of extracellular HIV-1 Tat protein that is a typical feature of E-KSC [23–26,39,102–105]. This leads to augmented angiogenesis and spindle cell growth in AIDS-KS [29]. IC also have proliferative and survival effects on both lesional and circulating KSC [21,39,106], and promote the differentiation of circulating KSC present in PBMC from patients with all forms KS or at risk of KS [28,52].

It should be pointed out, however, that non-tumorigenic E-KSC isolated from KS lesions show several traits that differentiate them from IC-activated EC. In



fact, E-KSC growth is stimulated by IC, whereas EC proliferation is inhibited by IC [96,106–109]. Furthermore, unlike EC, E-KSC produce large amounts of VEGF, but do not proliferate with VEGF despite the fact that the expression level of VEGF receptors is similar in these two cell types (Table 1) [27,40]. In addition, E-KSC, but not IC-activated EC, proliferate in response to RGD peptides [26] that bind to the  $\alpha 5\beta 1$  and  $\alpha v\beta 3$  integrins expressed by both IC-activated EC and E-KSC [25,26,29,102]. Our recent data also indicate that E-KSC are characterised by the constitutive activation of telomerase and matrix metalloproteinases, namely MMP-2 and MMP-9, that allow or mediate KSC proliferation and invasion, respectively (data not shown). In contrast, in normal EC these enzymatic activities are low or are found only upon treatment of cells with IC or angiogenic factors. These data indicate that E-KSC have acquired a “trans-differentiated” phenotype, although they are not transformed or tumorigenic. The phenotypic changes of E-KSC may reflect successive modifications intervening during the tumorigenic process. For example, we have recently obtained non-tumorigenic E-KSC clones that retain the typical EC markers but show a much higher proliferation rate with respect to most E-KSC cultures (data not shown). In addition, the expression of the HHV8 latency genes are likely to confer additional phenotypic features to KSC present in advanced lesions that it is not possible to reproduce in cultured primary E-KSC due to the loss of HHV8 from these cells upon culture [64,65].

#### 4.2. HHV8 reactivation by Th1-type cytokines, virus dissemination, and induction of immune responses exacerbating the KS reactive process

Several lines of evidence indicate that latent HHV8 is reactivated in individuals with KS or at risk of KS in response to IC. This, in turn, leads to virus spread in circulating cells and recruitment of infected cells into tissues.

Compared with HHV8-infected healthy individuals, patients with KS or at risk of KS show higher anti-HHV8 antibody titres and a high viral load in their PBMC, uninvolved tissues and body fluids including plasma, serum, nasal secretions, saliva, sperm or show evidence of HHV8 productive replication in their PBMC [20,21,28,88,91,110–117]. In particular, reactivation of HHV8 infection leading to PBMC-associated viraemia and high anti-latent or anti-lytic HHV8 antibody titres are frequently found in kidney transplant recipients [118,119] and HIV-1-infected individuals [28,78,84,85,88,91,112,113,117] that are at a high risk of KS. Evidence indicates that HHV8 load and viraemia are increased in these subjects before KS onset [87–91], as are the Th-1 type cytokines that are found to be increased in sera and tissues from individuals at risk of

KS even prior to KS development (Table 1) [20,21,67,69] (data not shown).

A link between IC and HHV8 infection in at risk individuals has been established by recent work showing that the same IC that are increased in KS reactivate latent HHV8. Evidence for this comes from the observation that PBMC from KS patients lose the virus upon culture, but maintain HHV8 DNA or show an increased HHV8 DNA load and activation of HHV8 lytic gene expression upon culture with IC (Fig. 3) [28].  $\gamma$ IFN appears to be key for these effects, although other IC may contribute to it (Fig. 3) [28]. These findings are supported by recent data indicating that recombinant IC or IC from HIV-1-infected cells including  $\gamma$ IFN, IL-1 $\beta$ , oncostatin M or scatter factor/hepatocyte growth factor (SF/HGF) increase the expression of HHV8 lytic genes also in chronically infected PEL-derived cell lines (Table 1). Since  $\gamma$ IFN and other Th1-type cytokines are produced at high levels during HIV-1 infection, these data may explain why progression to KS upon HHV8 infection is faster in the setting of HIV-1 infection compared with HIV-1-negative individuals [87,89].

Upon virus reactivation, HHV8 spreads to all circulating cell types. In fact, HHV8 DNA is found in B cells from normal donors, however, in patients with KS also CD4<sup>+</sup> and CD8<sup>+</sup> T cells and monocytes are infected [28,113,120–122] (data not shown). Infection of monocytes and T cells, in turn, is likely to be required for virus dissemination, in fact, productively-infected lympho-monocytes are found in KS lesions as they transmigrate through the activated endothelium [54,62]. However, it should be pointed out that tissue activation (i.e.  $\gamma$ IFN and HLA-DR expression) could be detected in few initial lesions and uninvolved tissues even prior to HHV8 detection, indicating that HHV8 exacerbates KS reactive processes, but may not initiate KS lesions [20].

The factors responsible for the spread of HHV8 infection to all circulating cell types are not completely understood. However, these likely include both the increased availability of infectious virus due to HHV8 reactivation by IC and the lack of immunological control of HHV8 infection. In fact, in addition to the overt immune suppression that is found in AIDS and PT patients, a declining trend in CD4<sup>+</sup> T cell counts is found in patients with C-KS and A-KS [71,76] that may impair immune responses against HHV8. Our recent data, moreover, indicates the presence of decreased natural killer (NK) cell cytotoxic responses in patients with KS compared with matched controls [74]. In this context, recent studies have also shown that recombinant  $\alpha$ IFN is a potent inhibitor of HHV8 reactivation in cultured PBMC from patients with KS or at risk of KS [117], raising the question of whether the integrity of this natural response to HHV8 infection is hampered in patients with KS or at risk of KS.

Altogether, these results indicate that virus reactivation and a variable degree of immunosuppression leading to virus spread and dissemination may be present in different settings or stages of KS development. In addition, recent data points to specific host-mediated and virus-encoded mechanisms of immune evasion as important factors allowing the dissemination of HHV8 infection. In fact, both HHV8-specific cytotoxic T lymphocytes (CTLs) and T helper cells response are found in peripheral blood from KS patients [123,124] and KS lesions themselves are infiltrated by numerous activated (i.e.  $\gamma$ IFN-producing) CD4<sup>+</sup> and CD8<sup>+</sup> T cells and NK cells [20,21]. However, these cells are unable to clear HHV8-infected cells from the circulation or tissues. This appears to be due, at least in part, to the expression of HHV8 open reading frames (ORFs) K13/v-FLIP, K3 and K5, whose gene products may allow HHV8-infected cells to escape CTL and NK cell responses through inhibition of the apoptotic FAS pathway or by down-regulating major histocompatibility complex class-I (MHC-I) molecules and co-activation molecules, respectively [58,125–129]. In addition, our recent data indicate that killer cell inhibitory receptors (KIRs) are

upregulated both in T cells and NK cells from KS patients or HIV-infected individuals without KS, suggesting that this is another mechanisms of altered immune control of HHV8 infection that may contribute to KS development and KS lesion growth in the absence of a manifest immunosuppression [75].

#### 4.3. Induction of angiogenic and chemotactic factors by Th1-type cytokines

IC induce infiltrating inflammatory cells, EC, and/or KSC to produce angiogenic molecules, growth factors and chemokines, including basic fibroblast growth factor (bFGF) and VEGF [20,27,40–42], SF/HGF and PDGF-B [130], monocyte chemotactic protein-1 (MCP-1) and IL-8 [131] (Table 1). These molecules are produced at high levels in all lesion stages and mediate KSC growth, angiogenesis, and oedema. In addition, they recruit circulating cells that transmigrate through the activated endothelia.

Both bFGF and VEGF are present at high levels in sera from patients with KS or at risk of KS and are highly expressed in KS lesions (Table 1) [27,40]. Evi-

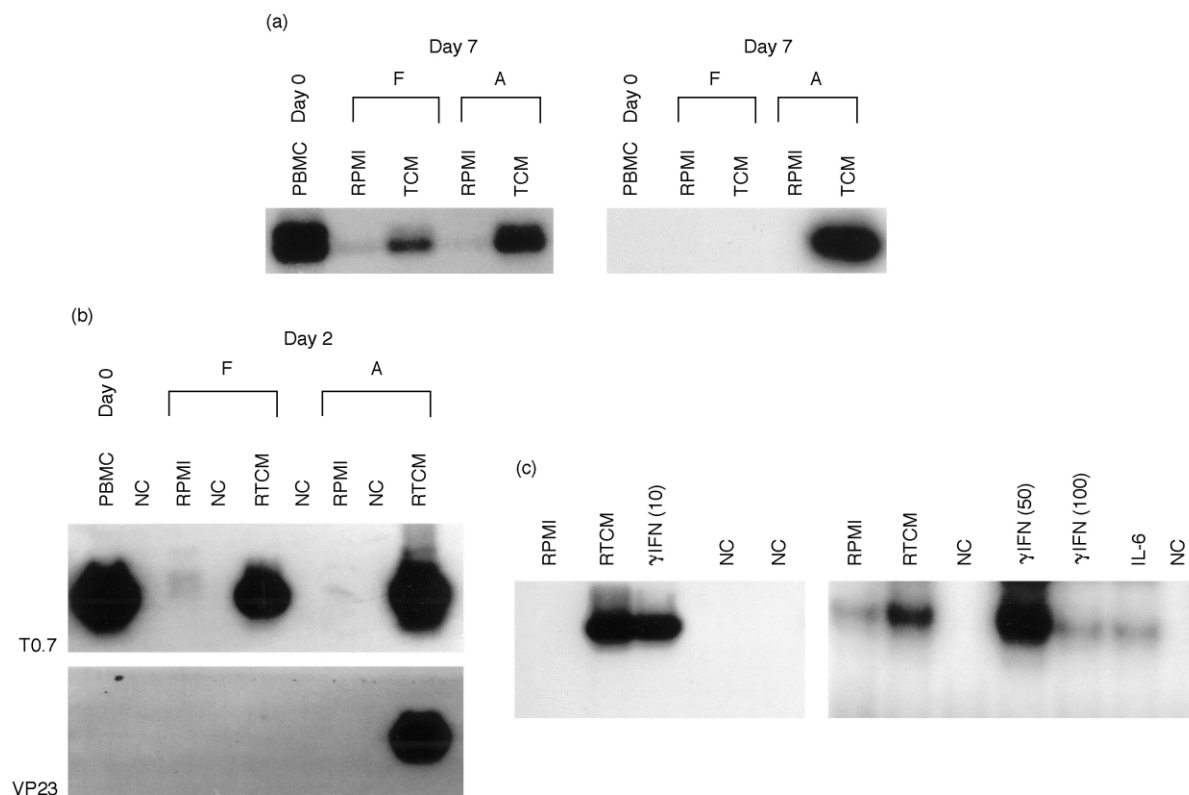


Fig. 3. Reactivation of HHV8 in PBMC from AIDS-KS patients by IC. (a) PCR detection of HHV8 DNA in PBMC (day 0) and in floating (F) or adherent (A) cells after culture for 7 days in the presence of IC (TCH or RTCM) or in their absence (RPMI). IC maintained or increased HHV8 to detectable levels in the floating or adherent cells. (b) HHV8 gene expression (RT-PCR) in PBMC at day 0 and in floating (F) or adherent (A) cells from a patient with AIDS-KS. The figure shows expression a viral latency transcript (T0.7) that is upregulated upon virus reactivation, or a transcript expressed only in productively-infected cells (VP23). (c) Effect of  $\gamma$ IFN on HHV8 reactivation. HHV8 DNA was detected or viral load increased after short-term culture of PBMC from 2 AIDS-KS patients only in the presence of RTCM or  $\gamma$ IFN. IL-6 showed little or no effect compared with standard medium (RPMI). NC, negative controls made without template DNA (modified with permission from Monini and colleagues [28] and The American Society of Hematology).

dence indicates that these angiogenic factors are the major mediators of KSC growth, angiogenesis and oedema present in KS. In particular, inhibition studies with neutralising antibodies or antisense oligodeoxynucleotides directed against bFGF have shown that bFGF is the main autocrine and paracrine growth factor for KSC, and this angiogenic factor is necessary and sufficient for the formation of KS lesions (Table 1) [97].

bFGF, that is released by KSC and IC-activated EC by a leaderless secretion pathway [20,27,41,42,96], has autocrine and paracrine growth and chemotactic activities, and stimulates angiogenesis (Table 1) [27,29,41,42,96]. Due to bFGF production, E-KSC or IC-activated EC are highly angiogenic in both the chorio-allantoic membrane or in nude mice, where they induce angiogenic lesions of mouse cell origin that closely resemble early KS [20,23,27,29,41,42,96,97]. In addition, the inoculation of recombinant bFGF in nude mice results in the formation of KS-like lesions [27,29]. The expression of bFGF is detected at both the RNA and protein level in both primary KS lesions, KS-like mice lesions, and in primary E-KSC or IC-activated EC (Table 1) [20,23,27,29,39,41,42,96].

VEGF is also produced by cultured KSC and synergises with bFGF in inducing EC growth, angiogenesis and oedema [27,40]. VEGF mediates the growth of two transformed KS cell lines established from KS lesions, and is produced in large amounts by E-KSC (Table 1). However, primary E-KSC do not proliferate in response to VEGF, despite the fact that the VEGF receptors KDR/FLK-1 and flt-1 are expressed by these cells *in vitro* and *in vivo* (Table 1) [27,40].

Other molecules with angiogenic or growth properties expressed in KS lesions include SF/HGF and PDGF-B, whose cognate receptors are also expressed *in vivo*, namely by KSC (Table 1) [38,130]. SF/HGF induces endothelial cells to acquire a spindle morphology and stimulates proliferation of cultured E-KSC (Table 1). PDGF-B is a potent E-KSC mitogen [38] and is expressed by cells intermingled with the spindle cells (Table 1) [38].

IC produced in KS lesions also induces the production of several chemokines including the monocyte chemoattractant protein-1 (MCP-1) and IL-8, that are expressed by E-KSC or IC-activated EC *in vitro* and *in vivo* and lead to the recruitment of monocytes into KS lesions (Table 1) [131]. In addition, other chemokines including the IFN- $\gamma$ -inducible protein 10 (IP-10) and Mig have been recently detected in inflammatory cells infiltrating KS lesions, in EC, and in KSC by immunohistochemistry analysis of AIDS-KS lesions (data not shown), and are most likely expressed in response to  $\gamma$ IFN that is produced in large amounts in KS lesions (Table 1). Finally, the MIP-1 $\alpha$  and  $\beta$  and RANTES (regulated upon activation, normal T cell expressed and secreted) are expressed by tumour-infiltrating lymphocytes and

PBMC from KS patients and are increased in sera from patients at risk of KS (Table 1) (data not shown). All these chemokines are likely to mediate the cell recruitment effects of IC or modulate angiogenesis, and may thus contribute to KS lesion development and growth.

## 5. KS progression

Although early stage KS is a reactive process and is polyclonal in nature, in time it can evolve into a monoclonal tumour. Evidence indicates that KS progression occurs upon the deregulated expression of anti-apoptotic genes (*Bcl-2*), oncogenes (*c-myc*, *c-int*, *ras*) and oncosuppressor genes (*TP53*) (Table 2), and is associated with the long-lasting expression of HHV8 latency genes (*LANA*, *v-cyc D*, *v-FLIP*, *Kaposin*). All these genes are, in fact, expressed or altered in most KSC in the nodular-late stage of KS. In addition, as discussed below, the HIV-1 Tat protein acts as a progression factor for AIDS-KS by stimulating KSC growth and angiogenesis. Evidence also indicates that a profound immunodeficiency may be required for progression of KS to a real sarcoma, that may be more common in AIDS-KS patients particularly from Africa [19], as also suggested by the microsatellite instability detected in AIDS-KS, but not in CKS [17].

### 5.1. Oncogenes and oncosuppressor genes

*Bcl-2*, an anti apoptotic protein, is expressed in lesional EC and KSC and its expression increases with lesion stage in all forms of KS (Table 2) [132]. *Bcl-2* acts as a major KS progression factor as indicated by KS regression in patients treated with paclitaxel that is known to inhibit *Bcl-2* function [133]. In fact, recent data indicates that paclitaxel blocks KSC growth and migration and KS-like lesion formation induced by the inoculation of KSC in nude mice due to a down-regulation of *Bcl-2* expression that leads to the apoptotic death of KS cells (Fig. 4) [134]. Several factors expressed in KS may contribute to *Bcl-2* activation. Among the IC increased in KS,  $\gamma$ IFN is known to upregulate in cultured E-KSC the expression of CD40 that is expressed by KSC and, in turn, is able to induce the expression of *Bcl-2*. In addition, recent data indicate that bFGF alone or combined with Tat inhibits EC apoptotic death by upregulating *Bcl-2* expression (B. Ensoli, data not shown). All these data suggest that *Bcl-2* expression allows the continued cell proliferation promoted by growth factors present in KS lesions leading to genetic instability and transformation of KS into a monoclonal tumour.

Among the oncosuppressor genes and oncogenes that have been associated with KS, evidence indicates that *TP53* and *c-myc* may have a role in disease progression.

In fact, heterozygous mutations and overexpression of p53 have been detected in late stage KS lesions, but not in early lesions (Table 2) and, as discussed below, recent data point to functional inactivation of p53 by HHV8 LANA as a mechanism to prevent the apoptosis of KSC (Table 2). In addition, c-myc is upregulated in KSC by PDGF-B and is expressed at higher levels in late-nodular KS lesions compared with early lesions (Table 2).

### 5.2. HHV8 latency genes

HHV8 latency gene products including v-Cyc D, v-FLIP, LANA and kaposin may be involved in KS progression due to their capability of promoting cell growth by direct effects or antiapoptotic effects, in fact, although few KSC appear to express these genes in early stage KS, their expression increases with lesion stage [43,56,58–60]. By contrast, the newly identified HHV8

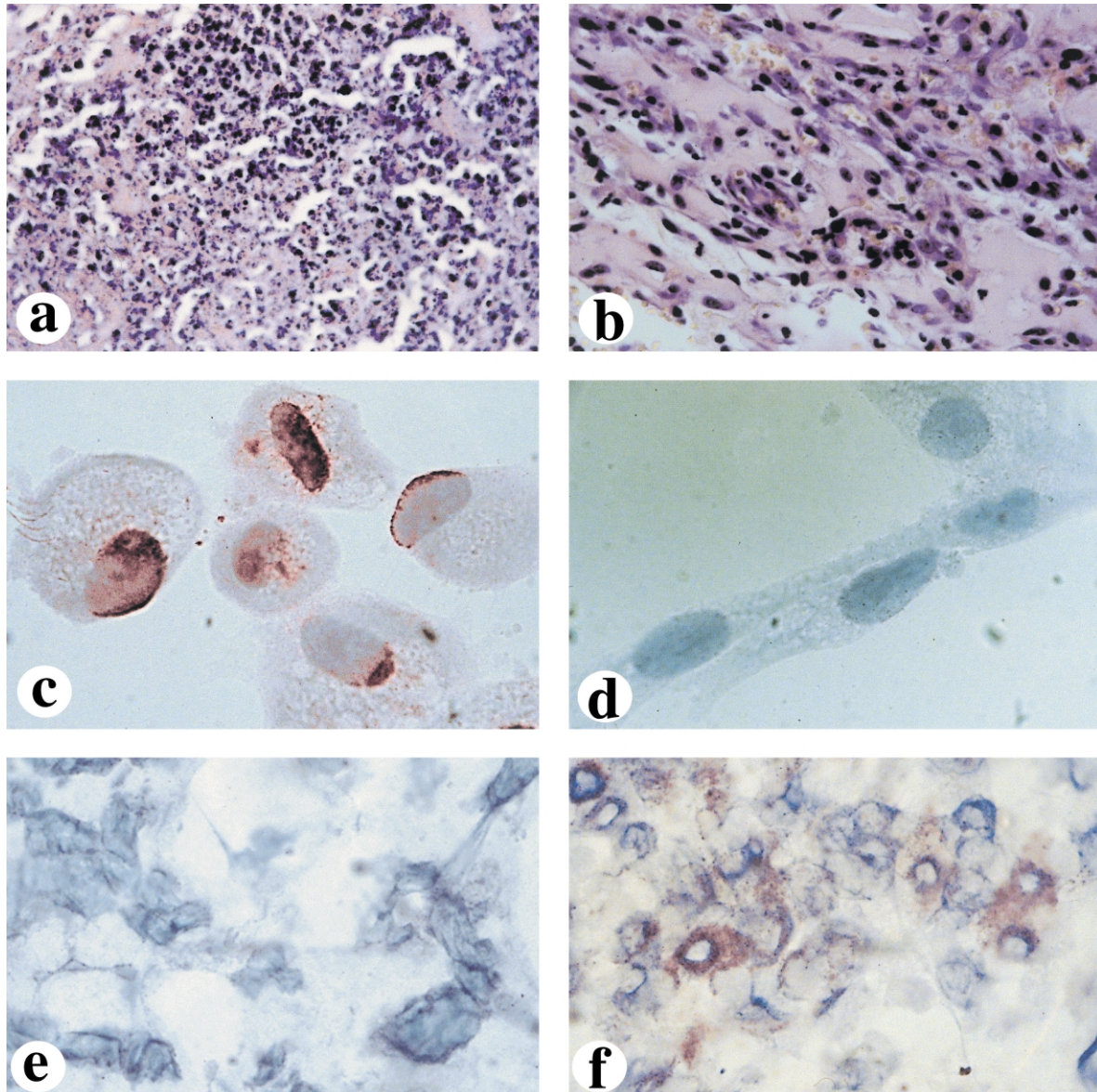


Fig. 4. Regression of experimental KS lesions in nude mice by paclitaxel due to Bcl-2 downregulation and KSC apoptosis. Upper panels: (a) regression of mouse KS-like lesions upon paclitaxel treatment. After 6 days of treatment, lesions from paclitaxel-treated mice present a central regressing area characterised by cells with picnotic nuclei, inflammatory cell infiltration (macrophages and polymorphonucleated cells) and absence of vessels. (b) No signs of regression are visible in control lesions (H&E staining, 40× magnification). Middle panels: Nuclei from human E-KSC cultured for 24 h with paclitaxel (c) appear rounded and show the typical perinuclear TUNEL staining indicating apoptosis. By contrast, control KSC (d) are negative for the TUNEL reaction and show the characteristic spindle morphology (100× magnification). Lower panels: Bcl-2 protein expression is almost completely down-modulated in lesions from paclitaxel-treated animals (e) compared with lesions from control animals (f) where numerous Bcl-2-positive cells are visible (APPAP method, 100× magnification) (modified with permission from Sgadari and colleagues [134]).

latency gene LANA 2 is not expressed in KS lesions [135].

v-cyc D is likely to play a role in KS progression due to effects on cell growth. In fact, this viral protein mediates phosphorylation of the retinoblastoma gene product (Rb) in a cdk-inhibitor-independent manner and downregulates p27/Kip 1. Consistent with these features, overexpression of v-cyc D induces cell cycle progression (Table 2).

The HHV8 *kaposin* locus may be involved in the progression of KS to the nodular-tumour stage by transforming KSC. This is suggested by the increased expression of *kaposin* transcripts in nodular lesions compared with early lesions [57] and by the capability of kaposin A to transform rodent cells (Table 2). However, our recent data indicate that kaposin A may not be capable of transforming human endothelial cells (data not shown).

v-FLIP may inhibit KSC apoptosis and allow KSC to escape CTL responses by interfering with the recruitment of the apoptotic FLICE driven by the TNF receptor family members (Table 2). In fact, expression of v-FLIP in late-nodular lesions correlates with a reduction in the apoptosis of KSC [58] and overexpression of v-FLIP in a murine B lymphoma cell line was shown to result in the growth of aggressive tumours in syngenic mice due to the inhibition of FAS-mediated cytotoxic T cell responses (Table 2).

HHV8 LANA is another HHV8 latency gene product that may contribute to the survival or transformation of KSC by the inactivation and downregulation of p53 and by targeting the Rb (Table 2). In addition, LANA has been shown to modulate host gene expression (Table 2).

Altogether, these data support the concept that HHV8 latency genes may play a key role in the progression of KS by providing KSC with growth and/or anti-apoptotic signals.

### 5.3. HIV-1 Tat protein

In HIV-1-infected individuals, the HIV-1 Tat protein appears to increase both the incidence and aggressiveness of KS by releasing bFGF bound to the ECM or cell membrane heparan sulphate proteoglycans (HPSG), and by delivering to KSC an adhesion signal that mediates their survival and growth.

Tat is a transcriptional activator of HIV-1 gene expression that becomes extracellular upon its release by HIV-1 acutely infected T cells in the absence of cell death [103–105]. In AIDS-KS lesions, extracellular Tat is found to be present in migrating inflammatory cells, EC lining vessels, and KSC (Fig. 5) where it co-stains with  $\alpha_5\beta_1$  and  $\alpha_v\beta_3$  integrins that function as receptors for Tat (see below) [29]. Extracellular Tat is biologically active and it can induce the adhesion growth, migration and invasion of cultured E-KSC [26,39,102,104,105]. Tat

exerts these effects also on normal EC that, however, become responsive to Tat only upon exposure to the same IC that are increased in KS [20,23,24,26,39,102]. In fact, Tat acts by binding the  $\alpha_5\beta_1$  and  $\alpha_v\beta_3$  integrins that are constitutively expressed by E-KSC, but are induced in EC upon activation by these IC [23–26,102]. In addition, IC induce EC and KSC to produce and release bFGF that, in turn, amplifies the expression of  $\alpha_v\beta_3$  integrins (Fig. 6) [25,27,41,42].

Recent data indicate that the angiogenic effects of Tat are due to the cooperation of Tat RGD sequence and

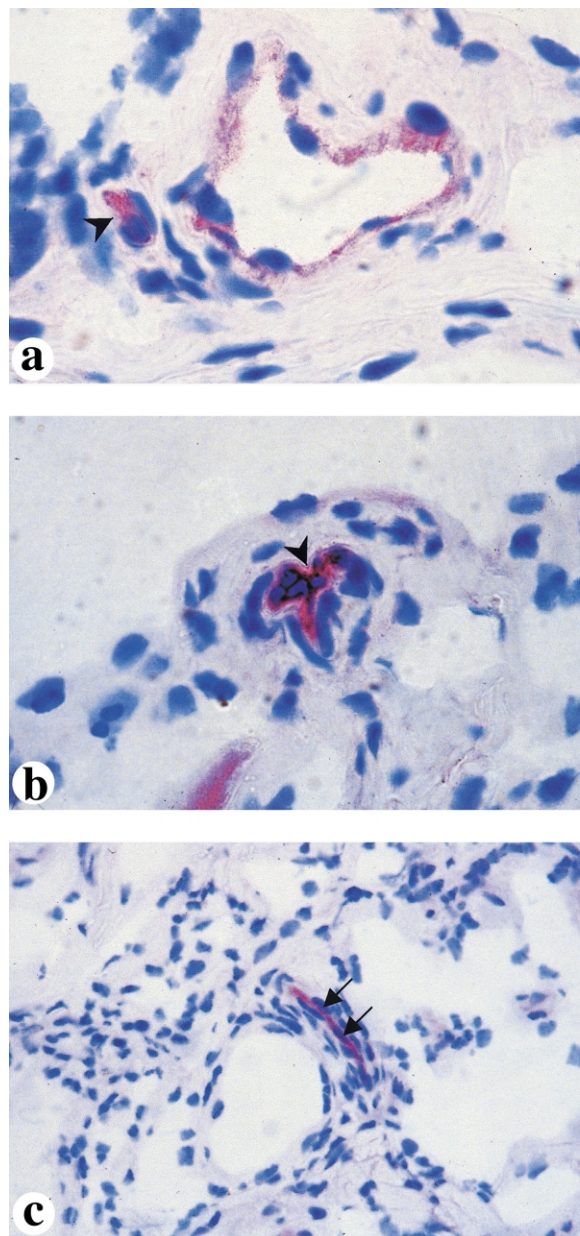


Fig. 5. Immunohistochemical detection of HIV-1 Tat in a AIDS-KS lesion. Tat is found to be expressed by inflammatory cells infiltrating the lesion (a and b, arrow-heads), normal endothelial cells lining a vessel (a) and spindle-shaped cells adjacent to a lesional vessel (c) (immunohistochemistry, APAAP, 100× magnification).

basic region that act by different pathways [26]. Specifically, by binding to the  $\alpha_5\beta_1$  and  $\alpha_v\beta_3$  integrins, the RGD sequence of Tat mediates EC adhesion, migration and invasion, and also provides EC with the adhesion

signal required for growth in response to mitogens. Consistent with these data, the development of KS-like lesions in mice is inhibited by RGD peptides that block the interaction of Tat with  $\alpha_5\beta_1$  and  $\alpha_v\beta_3$  integrins

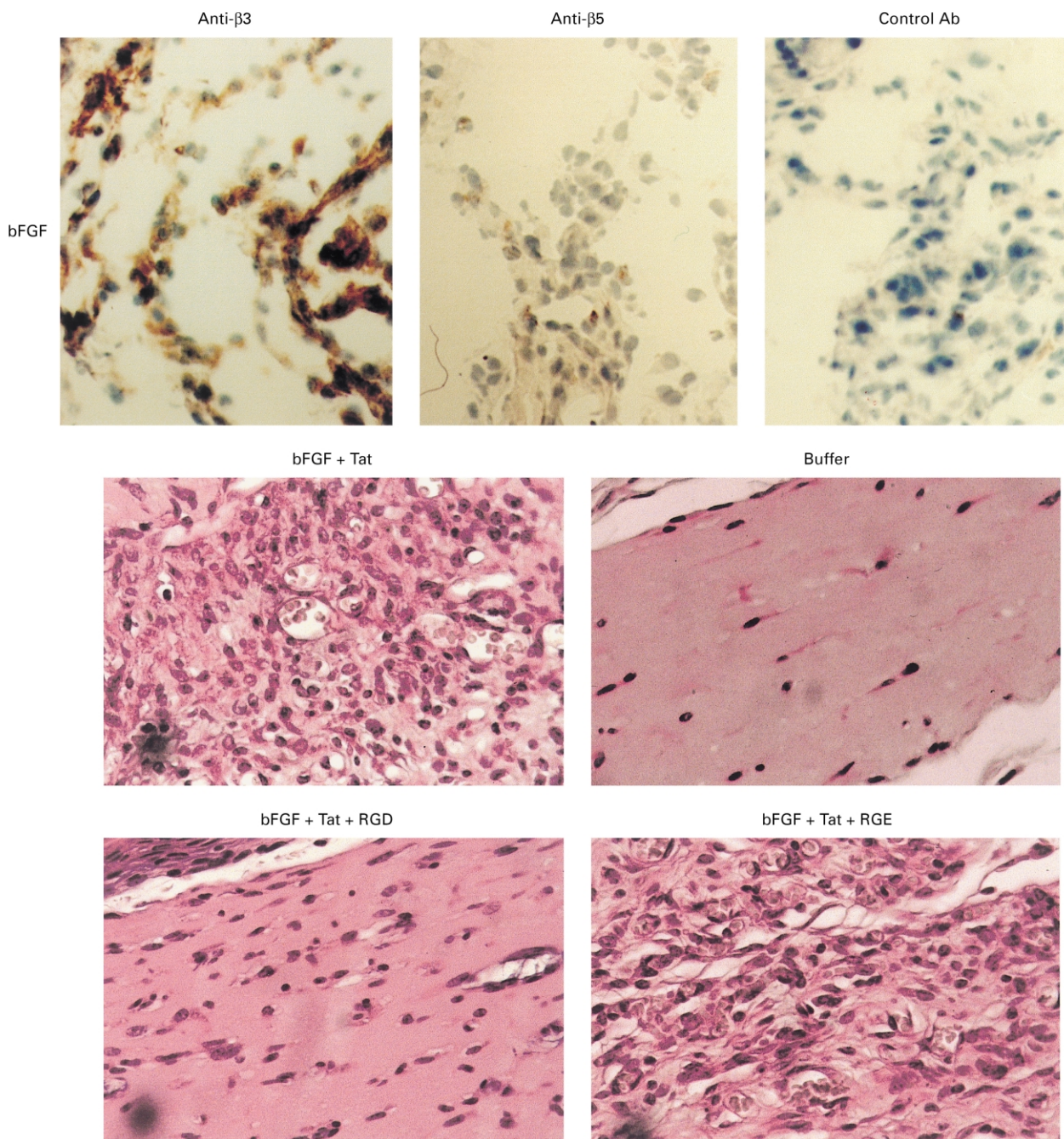


Fig. 6. Activation of  $\beta_3$  integrin expression by bFGF and synergistic effects of bFGF and Tat on the induction of KS-like lesions in mice. Upper panels: bFGF induces the  $\beta_3$  integrin (upper left) that functions as receptor for Tat, explaining the synergistic effects of Tat and bFGF on angiogenesis and KS (see lower panels). bFGF does not induce the  $\beta_5$  integrin (upper middle panel) that, in contrast, is induced at high levels by VEGF [25] (PAP method, 40 $\times$  magnification); VEGF, however, due to the lack of induction of  $\beta_3$ , does not synergise with Tat. Lower panels: Animals inoculated with Tat and bFGF combined (bFGF + Tat) develop KS-like lesions characterised by angiogenesis and spindle cell growth that are not visible in control tissue (Buffer) and are inhibited by the simultaneous inoculation of RGD peptides competing with Tat for binding to  $\alpha_5\beta_1$  and  $\alpha_v\beta_3$  integrins (bFGF + Tat + RGD), but not by RGE peptides lacking the RGD motif (bFGF + Tat + RGE). Animals do not develop macroscopic lesions upon inoculation of Tat or bFGF alone at the doses used (H&E staining, 40 $\times$  magnification) (modified with permission from Barillari and colleagues [25]).

(Fig. 6) [25]. Moreover, in a recent study it was shown that transgenic mice expressing HIV-1 Tat develop larger and more severe KS-like lesions upon inoculation of a KS-derived endothelial cell line compared with mice transgenic for a RGD-deleted Tat [136]. In turn, the Tat basic sequence competes for heparin-binding sites with extracellular bFGF bound to HPSG, retrieving it into a soluble form [26,103]. This soluble bFGF mediates Tat-induced vascular cell growth [26], explaining why bFGF is required for the angiogenic effect of Tat [29]. These data provide a molecular basis for previous studies showing that KS lesion formation in nude mice can be triggered in a synergistic way by bFGF and Tat [29]. Evidence that these mechanisms are operative *in vivo* comes from data indicating that  $\alpha_5\beta_1$  and  $\alpha_v\beta_3$  integrins and bFGF are highly expressed by KSC and activated EC of primary lesions and, as is the case for extracellular Tat, are present in AIDS-KS lesions (Fig. 5) [29].

Another mechanism by which Tat may affect KS development is through the induction of cytokines and adhesion molecules involved in KS pathogenesis, and MMPs mediating EC and KSC invasion. For example, Tat induces TNF $\alpha$  and  $\beta$ , IL-6 and other genes in infected cells (Table 2), and activates ELAM-1 expression in EC and V-CAM-1, I-CAM-1, MCP-1 and IL-6 in E-KSC (Table 2). MMP, and particularly MMP-2 and MMP-9, are highly expressed by KS cells both *in vitro* and in AIDS-KS lesions [26,29] (data not shown), and are induced by bFGF and further increased by Tat (Tables 1 and 2). Upregulation of MMPs by Tat may be necessary for the promotion of KS progression in the setting of HIV infection, in fact, these MMPs mediate vascular and KS cell invasion and vascular permeability *in vivo* and are essential for angiogenesis to occur.

## 6. Concluding remarks

The data reviewed in this article suggest that KS starts as an inflammatory-angiogenic process initiated by Th1-type cytokines that are increased at the systemic and tissue level even prior to disease development. IC activate vessels, leading to the appearance of foci of 'activated' tissue that may represent tissue sites prone to KS lesion development. In fact, circulating cells, including HHV8-infected cells and KSC progenitors, are recruited at these 'activated' sites, and differentiate in macrophages, dendritic cells and KSC with the endothelial-macrophages phenotype. These cells, in turn, produce other IC, chemokines and angiogenic factors, establishing a tissue microenvironment that leads to further cell recruitment and to the appearance of KSC of endothelial and macrophagic origin. These appear to be 'trans-differentiated' cells due to the acquisition of specific

phenotypic and functional traits such as the lack of proliferative response to VEGF, the capability of proliferating in response to RGD peptides, and constitutive telomerase and matrix metalloprotease activity. IC produced in KS lesions promote the growth and survival of KSC and induce the expression of angiogenic and growth factors such as bFGF and VEGF that mediate KS lesion development and oedema.

At the same time, the same IC reactivate HHV8 in circulating lymphocytes and monocytes, leading to HHV8-cell-associated viraemia that, like IC production, precedes KS development. A decreased NK cell cytotoxic activity, virus-escape mechanisms, or a more compromised immune response appear to favour the dissemination of HHV8 infection through the recruitment of infected cells at the 'activated' tissue sites. In this context, evidence indicates that HHV8 may spread in the circulation and tissues in response to the KS reactive processes, rather than initiate KS. In fact, IC and HLA-DR expression can be detected in KS lesions and uninvolved tissues prior to HHV8 detection.

HHV8 establishes a persistent, latent infection in KSC and lesional EC. Evidence indicates that this is likely to be accomplished through sequential and independent events of infection and/or re-infection upon the continuous exposure of KSC and EC to infectious virus released by cells infiltrating tissues. This is in fact indicated by the increase of viral load with lesion progression, the polyclonal or oligoclonal pattern of HHV8 TR found in most KS lesions, and by the loss of viral DNA upon culture of primary E-KSC. Thus, the cytokine milieu and the cell types found in KS lesions appear to be a unique and favourable microenvironment for virus infection.

HHV8-specific CTL and T helper responses found in KS patients and CD4<sup>+</sup> and CD8<sup>+</sup> T cells producing  $\gamma$ IFN present in KS lesions do not clear HHV8 infection from the tissues and circulation. Evidence suggests that this may be due to an upregulation of KIRs in T cells and NK cells from patients with KS or at risk of KS. However, expression of HHV8 gene products including K3, K5 and v-FLIP may favour immune evasion of KSC and other HHV8-infected cell types. These data indicate that HHV8 may play a role in KS initiation by inducing immune responses that, however, are not effective in controlling the virus and, paradoxically, exacerbate IC production and lesion growth.

Although KS initiates as a reactive process and is initially polyclonal, in time it can become monoclonal. This may occur upon the long-lasting expression of the HHV8 latency genes and the dysregulated expression of host oncogenes and oncosuppressor genes, and may be promoted by profound immune deficiency as suggested by the microsatellite instability detected in AIDS-KS, but not in CKS. The Tat protein of HIV-1 acts as a progression factor for AIDS-KS, and may be respon-

sible for the higher frequency and aggressiveness of KS in the setting of HIV-1 infection. In fact, Tat promotes KSC and IC-activated EC growth, migration and invasion by a molecular mimicry of the ECM proteins and by retrieving bFGF bound to the ECM or cell membrane HPSG. Furthermore, Tat induces the expression of cytokines, adhesion molecules and metalloproteinases that play a role in KS reactive processes or progression.

Thus, the efficacy of therapeutic intervention against KS relies on the capability of targeting key factors playing a role in the hyperplastic or tumour stage of KS, including IC, angiogenic factors, HHV8, HIV-1 Tat, molecules involved in the homing of circulating KSC, telomerase, metalloprotease, host and viral oncogenes or oncosuppressor genes.

### Acknowledgements

The authors' research was funded by AIDS grants from the Italian Ministry of Health and by grants from the Associazione Italiana per la Ricerca contro il Cancro (AIRC) to B. Ensoli, and by the Biofuture programme of the German Ministry of Education and Research (BMBF) and the Deutsche Krebshilfe (Dr Midred Scheel Stiftung) to M. Stürzl. We thank A. Lipa and A. Carinci for editorial assistance. We apologise for the studies on KS that could not be described or cited in this review due to restrictions on the length of the manuscript.

### References

- Geddes M, Franceschi S, Barchielli A, et al. Kaposi's sarcoma in Italy before and after the AIDS epidemic. *Br J Cancer* 1994, **69**, 333–336.
- Penn L. Kaposi's sarcoma in organ transplant recipients: report of 20 cases. *Transplantation* 1979, **27**, 8–11.
- Trattner A, Hodak E, David M, Sandbank M. The appearance of Kaposi's sarcoma during corticosteroid therapy. *Cancer* 1993, **72**, 1779–1783.
- Slavin G, Cameron HM, Singh H. Kaposi's sarcoma in mainland Tanzania: a report of 117 cases. *Br J Cancer* 1969, **23**, 349–357.
- Taylor JF, Smith PG, Bull D, Pike MC. Kaposi's sarcoma in Uganda: geographic and ethnic distribution. *Br J Cancer* 1972, **26**, 483–497.
- Friedman-Kien AE. Disseminated Kaposi's sarcoma syndrome in young homosexual men. *J Am Acad Dermatol* 1981, **5**, 468–471.
- Haverkos HW, Drotman DP. Prevalence of Kaposi's sarcoma among patients with AIDS. *N Engl J Med* 1985, **312**, 1518.
- Beral V, Peterman TA, Berkelman RL, Jaffe HW. Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? *Lancet* 1990, **335**, 123–128.
- Safai B, Johnson KG, Myskowski PL, et al. The natural history of Kaposi's sarcoma in the acquired immunodeficiency syndrome. *Ann Intern Med* 1985, **103**, 744–750.
- Brooks JJ. Kaposi's sarcoma: a reversible hyperplasia. *Lancet* 1986, **2**, 1309–1311.
- Janier M, Vignon MD, Cottenot F. Spontaneously healing Kaposi's sarcoma in AIDS. *N Engl J Med* 1985, **312**, 1638–1639.
- Lebbè C, Blum L, Pellet C, et al. Clinical and biological impact of antiretroviral therapy with protease inhibitors on HIV-related Kaposi's sarcoma. *AIDS* 1998, **12**, 45–49.
- Wit FWNM, Sol CJA, Renwick N, et al. Regression of AIDS-related Kaposi's sarcoma associated with clearance of human herpesvirus-8 from peripheral blood mononuclear cells following initiation of antiretroviral therapy. *AIDS* 1998, **1**, 218–219.
- Saikevych IA, Mayer M, White RL, Ho RC. Cytogenetic study of Kaposi associated with acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 1988, **112**, 825–828.
- Gill PS, Tsai YC, Rao AP, et al. Evidence for multiclonality in multicentric Kaposi's sarcoma. *Proc Natl Acad Sci USA* 1998, **15**, 8257–8261.
- Dictor M, Ferno M, Baldetorp B. Flow cytometric DNA content in Kaposi's sarcoma by histologic stage: comparison with angiosarcoma. *Anal Quant Cytol Histol* 1991, **13**, 201–208.
- Bedi GC, Westra WH, Farzadegan H, Pitha PM, Sidransky D. Microsatellite instability in primary neoplasms from HIV+ patients. *Nat Med* 1995, **1**, 65–68.
- Rabkin CS, Bedi G, Musaba E, et al. AIDS-related Kaposi's sarcoma is a monoclonal neoplasm. *Clin Cancer Res* 1995, **1**, 257–260.
- Rabkin CS, Janz S, Lash A, et al. Monoclonal origin of multicentric Kaposi's sarcoma lesions. *N Engl J Med* 1997, **336**, 988–993.
- Fiorelli V, Gendelman R, Sirianni MC, et al.  $\gamma$ -Interferon produced by CD8+ T cells infiltrating Kaposi's sarcoma induces spindle cells with angiogenic phenotype and synergy with HIV-1 Tat protein: an immune response to HHV-8 infection? *Blood* 1998, **91**, 956–967.
- Sirianni MC, Vincenzi L, Fiorelli V, et al.  $\gamma$ -Interferon production in peripheral blood mononuclear cells (PBMC) and tumour infiltrating lymphocytes from Kaposi's sarcoma patients: correlation with the presence of human herpesvirus-8 in PBMC and lesional macrophages. *Blood* 1998, **91**, 968–976.
- Zietz C, Hotz B, Stürzl M, Rauch E, Penning R, Lohrs U. Aortic endothelium in HIV-1 infection: chronic injury, activation, and increased leukocyte adherence. *Am J Pathol* 1996, **149**, 1887–1898.
- Fiorelli V, Gendelman R, Samaniego F, Markham PD, Ensoli B. Cytokines from activated T cells induce normal endothelial cells to acquire the phenotypic and functional features of AIDS-Kaposi's sarcoma spindle cells. *J Clin Invest* 1995, **95**, 1723–1734.
- Fiorelli V, Barillari G, Toschi E, Sgadari C, Monini P, Stürzl M, Ensoli B. IFN- $\gamma$  induces endothelial cells to proliferate and to invade the extracellular matrix in response to the HIV-1 Tat protein: implications for AIDS-Kaposi's sarcoma pathogenesis. *J Immunol* 1999, **162**, 1165–1170.
- Barillari G, Sgadari C, Palladino C, et al. Inflammatory cytokines synergize with the HIV-1 Tat protein to promote angiogenesis and Kaposi's sarcoma via induction of bFGF and  $\alpha$ v $\beta$ 3 integrin that are required for Tat activity. *J Immunol* 1999, **162**, 1165–1170.
- Barillari G, Sgadari C, Fiorelli V, et al. The Tat protein of human immunodeficiency virus type-1 promotes vascular cell growth and locomotion by engaging the  $\alpha$ 5 $\beta$ 3 integrins and by mobilizing sequestered basic fibroblast growth factor. *Blood* 1999, **94**, 663–672.
- Samaniego F, Markham PD, Gendelman R, et al. Vascular endothelial growth factor and basic fibroblast growth factor present in Kaposi's sarcoma are induced by inflammatory cytokines and synergize to induce vascular permeability and KS lesion development. *Am J Pathol* 1998, **152**, 1433–1443.



28. Monini P, Colombini S, Stürzl M, et al. Reactivation and persistence of human herpesvirus-8 infection in B cells and monocytes by Th-1 cytokines increased in Kaposi's sarcoma. *Blood* 1999, **93**, 4044–4058.
29. Ensoli B, Gendelman R, Markham P, et al. Synergy between basic fibroblast growth factor and human immunodeficiency virus type I Tat protein in induction of Kaposi's sarcoma. *Nature* 1994, **371**, 674–680.
30. Dorfman RF, Path FRC. The histogenesis of Kaposi's sarcoma. *Lymphology* 1984, **17**, 76–77.
31. MacPhail LA, Dekker NP, Regezi JA. Macrophages and vascular adhesion molecules in oral Kaposi's sarcoma. *J Cutaneous Pathol* 1996, **23**, 464–472.
32. Nickoloff BJ, Griffiths CE. Factor XIIIa-expressing dermal dendrocytes in AIDS-associated cutaneous Kaposi's sarcomas. *Science* 1989, **243**, 1736–1737.
33. Regezi JA, MacPhail LA, Daniels TE, DeSouza YG, Greenspan JS, Greenspan D. Human immunodeficiency virus-associated oral Kaposi's sarcoma. A heterogeneous cell population dominated by spindle-shaped endothelial cells. *Am J Pathol* 1993, **143**, 240–249.
34. Tabata M, Langford A, Becker J, Reichart PA. Distribution of immunocompetent cells in oral Kaposi's sarcoma (AIDS). *Eur J Cancer B Oral Oncol* 1993, **29**, 209–213.
35. Uccini S, Ruco LP, Monardo F, et al. Co-expression of endothelial cell and macrophage antigens in Kaposi's sarcoma cells. *J Pathol* 1994, **173**, 23–31.
36. Uccini S, Sirianni MC, Vincenzi L, et al. Kaposi's sarcoma (KS) cells express the macrophage associated antigen mannose receptor and develop in peripheral blood cultures of KS patients. *Am J Pathol* 1997, **150**, 929–938.
37. Miles SA, Rezaei AR, Salazar-Gonzales JF, et al. AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6. *Proc Natl Acad Sci USA* 1990, **87**, 4068–4072.
38. Stürzl M, Brandstetter H, Zietz C, et al. Identification of interleukin-1 and platelet-derived growth factor- $\beta$  as major mitogens for the spindle cells of Kaposi's sarcoma: a combined in vitro and in vivo analysis. *Oncogene* 1995, **10**, 2007–2016.
39. Barillari G, Buonaguro L, Fiorelli V, et al. Effects of cytokines from activated immune cells on vascular cell growth and HIV-1 gene expression: implications for AIDS-Kaposi's sarcoma pathogenesis. *J Immunol* 1992, **149**, 3727–3734.
40. Cornali E, Zietz C, Benelli R, et al. Vascular endothelial growth factor regulates angiogenesis and vascular permeability in Kaposi's sarcoma. *Am J Pathol* 1996, **149**, 1851–1869.
41. Samaniego F, Markham P, Gallo RC, Ensoli B. Inflammatory cytokines induce AIDS-Kaposi's sarcoma-derived spindle cells to produce and release basic fibroblast growth factor and enhance Kaposi's sarcoma-like lesion formation in nude mice. *J Immunol* 1995, **154**, 3582–3592.
42. Samaniego F, Markham PD, Gendelman R, Gallo RC, Ensoli B. Inflammatory cytokines induce endothelial cells to produce and release basic fibroblast growth factor and to promote Kaposi's sarcoma-like lesions in nude mice. *J Immunol* 1997, **158**, 1887–1894.
43. Dupin N, Fisher C, Kellam P, et al. Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. *Proc Natl Acad Sci USA* 1999, **96**, 4546–4551.
44. Kraffert C, Pianus L, Penneys NS. Kaposi's sarcoma: further immunohistologic evidence of a vascular endothelial origin. *Arch Dermatol* 1991, **127**, 1734–1735.
45. Jussila L, Valtola R, Partanen TA, et al. Lymphatic endothelium and Kaposi's sarcoma spindle cells detected by antibodies against the vascular endothelial growth factor receptor-3. *Cancer Res* 1998, **58**, 1599–1604.
46. Nadji M, Morales AR, Zigler-Weissman J, Pehneys NS. Kaposi's sarcoma: immunohistologic evidence for an endothelial origin. *Arch Pathol Lab Med* 1981, **105**, 274–275.
47. Pammer J, Plettenberg A, Weninger W, et al. CD40 antigen is expressed by endothelial cells and tumor cells in Kaposi's sarcoma. *Am J Pathol* 1996, **148**, 1387–1396.
48. Roth WK, Werner S, Risau W, Remberger K, Hofschneider PH. Cultured, AIDS-related Kaposi's sarcoma cells express endothelial cell markers and are weakly malignant in vitro. *Int J Cancer* 1998, **42**, 767–773.
49. Yang J, Xu Y, Zhu C, Hagan MK, Lawley T, Hoffermann MK. Regulation of adhesion molecular expression in Kaposi's sarcoma cells. *J Immunol* 1994, **152**, 361–373.
50. Weninger W, Partanen TA, Breiteneder-Geleff S, et al. Expression of vascular endothelial growth factor receptor-3 and podoplanin suggests a lymphatic endothelial cell origin of Kaposi's sarcoma tumor cells. *Lab Invest* 1999, **79**, 243–251.
51. Zhang YM, Bachmann S, Hemmer C, et al. Vascular origin of Kaposi's sarcoma: expression of leukocyte endothelial adhesion molecule-1, thrombomodulin, and tissue factor. *Am J Pathol* 1994, **144**, 51–59.
52. Browning PJ, Sechler JM, Kaplan M, et al. Identification and culture of Kaposi's sarcoma-like spindle cells from the peripheral blood of HIV-1-infected individuals and normal controls. *Blood* 1994, **84**, 2711–2720.
53. Sirianni MC, Uccini S, Angeloni A, Faggioni A, Cottoni F, Ensoli B. Circulating spindle cells: correlation with human herpesvirus-8 (HHV-8) infection and Kaposi's sarcoma. *Lancet* 1997, **349**, 225.
54. Blasig C, Zietz C, Haar B, et al. Monocytes in Kaposi's sarcoma lesions are productively infected by human herpesvirus 8. *J Virol* 1997, **71**, 7963–7968.
55. Boshoff C, Schulz TF, Kennedy MM, et al. Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. *Nat Med* 1995, **1**, 1274–1278.
56. Davis MA, Stürzl M, Blasig C, et al. Expression of human herpesvirus 8-encoded cyclin D in Kaposi's sarcoma spindle cells. *J Natl Cancer Inst* 1997, **89**, 1868–1874.
57. Stürzl M, Blasig C, Schreier A, et al. Expression of HHV-8 latency-associated T0.7 RNA in spindle cells and endothelial cells of AIDS-associated, classical and African Kaposi's sarcoma. *Int J Cancer* 1997, **72**, 68–71.
58. Stürzl M, Hohenadl C, Zietz C, et al. Expression of K13/v-FLIP gene of human herpesvirus 8 and apoptosis in Kaposi's sarcoma spindle cells. *J Natl Cancer Inst* 1999, **91**, 1725–1733.
59. Stürzl M, Wunderlich A, Ascherl G, et al. Human herpesvirus-8 (HHV-8) gene expression in Kaposi's sarcoma (KS) primary lesions: an in situ hybridization study. *Leukemia* 1999, **13**, S110–S112.
60. Staskus KA, Zhong W, Gebhard K, et al. Kaposi's sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumor cells. *J Virol* 1997, **71**, 715–719.
61. Li M, Lee H, Yoon DW, et al. Kaposi's sarcoma-associated herpesvirus encodes a functional cyclin. *J Virol* 1997, **71**, 1984–1991.
62. Orenstein JM, Alkan S, Blauvelt A, et al. Visualization of human herpesvirus type 8 in Kaposi's sarcoma by light and transmission electron microscopy. *AIDS* 1997, **11**, 35–45.
63. Moore PS, Boshoff C, Weiss RA, Chang Y. Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. *Science* 1996, **274**, 1739–1744.
64. Lebbè C, de Cremoux P, Rybojad M, et al. Kaposi's sarcoma and new herpesvirus. *Lancet* 1995, **345**, 1180.
65. Monini P, Rotola A, de Lellis L, et al. Latent BK virus infection and Kaposi's sarcoma pathogenesis. *Int J Cancer* 1996, **66**, 717–722.

66. Fagnoni FF, Vescovini R, Mazzola M, et al. Expansion of cytotoxic CD8+ CD28T cells in healthy ageing people, including centenarians. *Immunology* 1996, **88**, 501–507.
67. Honda M, Kitamura K, Mizutani Y, et al. Quantitative analysis of serum IL-6 and its correlation with increased levels of serum IL2R in HIV-I-induced disease. *J Immunol* 1990, **145**, 4059–4064.
68. Master SP, Taylor JF, Kyalwazi SK, Ziegler JL. Immunologic studies in Kaposi's sarcoma in Uganda. *Br J Med* 1970, **1**, 600–602.
69. Rizzardini G, Piconi S, Ruzzante S, et al. Immunological activation markers in the serum of African and European HIV-seropositive and seronegative individuals. *AIDS* 1996, **10**, 1535–1542.
70. Schlesinger M, Chu FN, Badamchian M, et al. A distinctive form of soluble CD8 is secreted by stimulated CD8+ cells in HIV-1-infected and high risk individuals. *Clin Immunol Immunopathol* 1994, **73**, 252–260.
71. Touloumi G, Hatzakis A, Potouridou I, et al. The role of immunosuppression and immune-activation in classic Kaposi's sarcoma. *Int J Cancer* 1999, **82**, 817–821.
72. Mitsuyasu RT. Clinical aspects of AIDS-related Kaposi's sarcoma. *Curr Opin Oncol* 1993, **5**, 835–844.
73. Friedman-Kien AE, Saltzman BR, Cao YZ, et al. Kaposi's sarcoma in HIV-negative homosexual men. *Lancet* 1990, **335**, 168–169.
74. Sirianni MC, Vincenzi L, Topino S, et al. Immune response to human herpesvirus-8 (HHV-8): study of natural killer phenotype and activity in Kaposi's sarcoma patients. In *2nd International Workshop on KSHV/HHV8 and Related Agents*, St Catherine's College, Oxford, UK, 10–13 September 1999.
75. Sirianni MC, Alario C, Libi F, et al. Expression of the natural killer-related receptor for HLA-C on natural killer and T lymphocytes from Kaposi's sarcoma patients. In *3rd International Workshop on Kaposi's Sarcoma-associated Herpesvirus and Related Agents*, Amherst, MA, USA, 6–10 June 2000.
76. Urassa WK, Kaaya EE, Kitinya JN, et al. Immunological profile of endemic and epidemic Kaposi's sarcoma patients in Dar-es-salaam, Tanzania. *Int J Mol Med* 1998, **1**, 979–982.
77. de-The G, Bestetti G, van Beveren M, Gessain A. Prevalence of human herpesvirus 8 infection before the acquired immunodeficiency disease syndrome-related epidemic of Kaposi's sarcoma in East Africa. *J Natl Cancer Inst* 1999, **91**, 1888–1889.
78. Gao SJ, Kingsley L, Li M, et al. KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. *Nat Med* 1996, **2**, 925–928.
79. Lennette ET, Blackbourn DJ, Levy JA. Antibodies to human herpesvirus type 8 in the general population and in Kaposi's sarcoma patients. *Lancet* 1996, **348**, 858–861.
80. Rezza G, Lennette ET, Giuliani M, et al. Prevalence and determinants of anti-lytic and anti-latent antibodies to human herpesvirus-8 among Italian individuals at risk of sexually and parenterally transmitted infections. *Int J Cancer* 1998, **77**, 361–365.
81. Rezza G, Tchamgema OB, Andreoni M, et al. Prevalence and risk factors for human herpesvirus 8 infection in northern Cameroon. *Sex Transm Dis* 2000, **27**, 159–164.
82. Simpson GR, Schulz TF, Whitby D, et al. Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. *Lancet* 1996, **349**, 1133–1138.
83. Whitby D, Luppi M, Barozzi P, Boshoff C, Weiss RA, Torelli G. Human herpesvirus 8 seroprevalence in blood donors and lymphoma patients from different regions of Italy. *J Natl Cancer Inst* 1998, **90**, 395–397.
84. Gao SJ, Kingsley L, Hoover DR, et al. Seroconversion to antibodies against Kaposi's sarcoma-associated herpesvirus-related latent nuclear antigens before the development of Kaposi's sarcoma. *N Engl J Med* 1996, **335**, 233–241.
85. Kedes DH, Operskalski E, Busch M, Kohn R, Flood J, Ganem D. The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. *Nat Med* 1996, **2**, 918–924.
86. Martin JN, Ganem DE, Osmond DH, Page-Shafer KA, Macrae D, Kedes DH. Sexual transmission and the natural history of human herpesvirus 8 infection. *N Engl J Med* 1998, **338**, 948–954.
87. Jacobson LP, Jenkins FJ, Springer G, et al. Interaction of human immunodeficiency virus type 1 and human herpesvirus type 8 infections on the incidence of Kaposi's sarcoma. *J Infect Dis* 2000, **181**, 1940–1949.
88. Moore PS, Kingsley LA, Holmberg SD, et al. Kaposi's sarcoma associated herpesvirus infection prior to onset of Kaposi's sarcoma. *AIDS* 1996, **10**, 175–180.
89. Renwick N, Halaby T, Weverling GJ, et al. Seroconversion for human herpesvirus 8 during HIV infection is highly predictive of Kaposi's sarcoma. *AIDS* 1998, **12**, 2481–2488.
90. Rezza G, Andreoni M, Dorrucchi M, et al. Human herpesvirus-8 seropositivity and risk of developing Kaposi's sarcoma and other AIDS-related diseases. *J Natl Cancer Inst* 1999, **91**, 1468–1474.
91. Whitby D, Howard MR, Tenant-Flowers M, et al. Detection of Kaposi's sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. *Lancet* 1995, **346**, 799–802.
92. Zisbrod Z, Haymov M, Schanzer H, Ambinder E, Burrows L. Kaposi's sarcoma after kidney transplantation. Report of complete remission of cutaneous and visceral involvement. *Transplantation* 1980, **30**, 383–384.
93. Judde JG, Lacoste V, Briere J, et al. Monoclonality or oligoclonality of human herpesvirus 8 terminal repeat sequences in Kaposi's sarcoma and other diseases. *J Natl Cancer Inst* 2000, **92**, 729–736.
94. Bisceglia M, Bosman C, Quirke P. A histologic and flow cytometric study of Kaposi's sarcoma. *Cancer* 1992, **69**, 793–798.
95. Kaaya EE, Parravicini C, Sundelin B, et al. Spindle cell ploidy and proliferation in endemic and epidemic African Kaposi's sarcoma. *Eur J Cancer* 1992, **28A**, 1890–1894.
96. Ensoli B, Nakamura S, Salahuddin SZ, et al. AIDS-Kaposi's sarcoma-derived cells express cytokines with autocrine and paracrine growth effects. *Science* 1989, **243**, 223–226.
97. Ensoli B, Markham P, Kao V, et al. Block of AIDS-Kaposi's sarcoma (KS) cell growth, angiogenesis, and lesion formation in nude mice by antisense oligonucleotide targeting basic fibroblast growth factor. A novel strategy for the therapy of KS. *J Clin Invest* 1994, **94**, 1736–1746.
98. Herndier BG, Werner A, Arnstein P, et al. Characterization of a human Kaposi's sarcoma cell line that induces angiogenic tumors in animals. *AIDS* 1994, **8**, 575–581.
99. Lunardi-Iskandar Y, Gill P, Lam VH, et al. Isolation and characterization of an immortal neoplastic cell line (KS Y-1) from AIDS-associated Kaposi's sarcoma. *J Natl Cancer Inst* 1995, **87**, 974–981.
100. Gariano RF, Rickman LS, Freeman WR. Ocular examination and diagnosis in patients with the acquired immunodeficiency syndrome. *West J Med* 1993, **158**, 254–262.
101. Petitto CK, Cash KS. Blood-brain barrier abnormalities in the acquired immunodeficiency syndrome: immunohistochemical localization of serum proteins in postmortem brain. *Ann Neurol* 1992, **32**, 658–666.
102. Barillari G, Gendelman R, Gallo RC, Ensoli B. The Tat protein of human immunodeficiency virus type 1, a growth factor for AIDS Kaposi's sarcoma and cytokine-activated vascular cells,

- induces adhesion of the same cell types by using integrin receptors recognizing the RGD amino acid sequence. *Proc Natl Acad Sci USA* 1993, **90**, 7941–7945.
103. Chang HC, Samaniego F, Nair BC, Buonaguro L, Ensoli B. HIV-1 Tat protein exits from cells via leaderless secretory pathway and binds to extracellular matrix-associated heparan sulfate proteoglycans through its basic region. *AIDS* 1997, **11**, 1421–1431.
  104. Ensoli B, Barillari G, Salahuddin SZ, Gallo RC, WongStaal F. The Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. *Nature* 1990, **345**, 84–86.
  105. Ensoli B, Buonaguro L, Barillari G, et al. Release, uptake, and effects of extracellular HIV-1 Tat protein on cell growth and viral transactivation. *J Virol* 1993, **67**, 277–287.
  106. Nakamura S, Salahuddin SZ, Biberfeld P, et al. Kaposi's sarcoma cells: long-term culture with growth factor from retrovirus-infected CD4<sup>+</sup> T cells. *Science* 1988, **242**, 426–430.
  107. Cozzolino F, Torcia M, Aldinucci D, et al. Interleukin 1 is an autocrine regulator of human endothelial cell growth. *Proc Natl Acad Sci USA* 1990, **87**, 6487–6491.
  108. Frater-Schroder M, Risau W, Hallmann R, Gautschi P, Bohlen P. Tumor necrosis factor type alpha, a potent inhibitor of endothelial cell growth in vitro, is angiogenic in vivo. *Proc Natl Acad Sci USA* 1987, **84**, 5277–5281.
  109. Friesel R, Komoriya A, Maciag T. Inhibition of endothelial cell proliferation by gamma-interferon. *J Cell Biol* 1987, **104**, 689–696.
  110. Blackbourn DJ, Lennette ET, Ambroziak J, Mourich DV, Levy JA. Human herpesvirus 8 detection in nasal secretions and saliva. *J Infect Dis* 1998, **177**, 213–216.
  111. Corbellino M, Poirer L, Bestetti G, et al. Restricted tissue distribution of extralosomal Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS patients with Kaposi's sarcoma. *AIDS Res Hum Retroviruses* 1996, **12**, 651–657.
  112. Decker LL, Shankar P, Khan G, et al. The Kaposi sarcoma-associated herpesvirus (KSHV) is present as an intact latent genome in KS tissue but replicates in the peripheral blood mononuclear cells of KS patients. *J Exp Med* 1996, **184**, 283–288.
  113. Harrington WJ, Bagasra O, Sosa CE, et al. Human herpesvirus type 8 DNA sequences in cell-free plasma and mononuclear cells of Kaposi's sarcoma patients. *J Infect Dis* 1996, **174**, 1101–1105.
  114. Howard MR, Whitby D, Bahadur G, et al. Detection of human herpesvirus 8 DNA in semen from HIV-infected individuals but not healthy semen donors. *AIDS* 1997, **11**, F15–F19.
  115. Koelle DM, Huang ML, Chandran B, Vieira J, Piepkorn M, Corey L. Frequent detection of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) DNA in saliva of human immunodeficiency virus-infected men: clinical and immunologic correlates. *J Infect Dis* 1997, **176**, 94–102.
  116. LaDuca JR, Love JL, Abbott LZ, Dube S, Freidman-Kien AE, Poesz BJ. Detection of human herpesvirus 8 DNA sequences in tissues and bodily fluids. *J Infect Dis* 1998, **178**, 1610–1615.
  117. Monini P, Carlini F, Stürzl M, et al.  $\alpha$ -Interferon inhibits HHV-8 reactivation in primary effusion lymphoma cells and reduces HHV-8 load in cultured PBMC. *J Virol* 1999, **73**, 4029–4041.
  118. Hudnall SD, Rady PL, Tyring SK, Fish JC. Serologic and molecular evidence of human herpesvirus 8 activation in renal transplant recipients. *J Infect Dis* 1998, **178**, 1791–1794.
  119. Mendez JC, Procop GW, Espy MJ, Smith TF, McGregor CG, Paya CV. Relationship of HHV8 replication and Kaposi's sarcoma after solid organ transplantation. *Transplantation* 1999, **67**, 1200–1201.
  120. Ambroziak JA, Blackbourn DJ, Herndier BG, et al. Herpes-like sequences in HIV-infected and uninfected Kaposi's sarcoma patients. *Science* 1995, **268**, 582–583.
  121. Howard MR, Brink NS, Whitby D, Tedder RS, Miller RF. Association of Kaposi's sarcoma associated herpesvirus (KSHV) DNA in bronchoalveolar lavage fluid of HIV infected individuals with bronchoscopically diagnosed tracheobronchial Kaposi's sarcoma. *Sex Transm Infect* 1998, **74**, 27–31.
  122. Sirianni MC. Human herpesvirus-8 DNA sequences in CD8<sup>+</sup> T cells. *J Infect Dis* 1997, **176**, 541.
  123. Osman M, Kubo T, Gill J, et al. Identification of human herpesvirus 8-specific cytotoxic T-cell responses. *J Virol* 1999, **73**, 6136–6140.
  124. Strickler HD, Goedert JJ, Bethke FR, et al. Human herpesvirus 8 cellular immune responses in homosexual men. *J Infect Dis* 1999, **180**, 1682–1685.
  125. Coscoy L, Ganem D. Kaposi's sarcoma-associated herpesvirus encodes two proteins that block cell surface display of MHC class I chains by enhancing their endocytosis. *Proc Natl Acad Sci USA* 2000, **97**, 8051–8056.
  126. Haque M, Chen J, Ueda K, et al. Identification and analysis of the K5 gene of Kaposi's sarcoma-associated herpesvirus. *J Virol* 2000, **74**, 2867–2875.
  127. Ishido S, Wang C, Lee B-S, Cohen GB, Jung JU. Down-regulation of major histocompatibility complex class I molecules by Kaposi's sarcoma-associated herpesvirus K3 and K5 proteins. *J Virol* 2000, **74**, 5300–5309.
  128. Ishido S, Choi J-K, Lee B-S, et al. Inhibition of natural killer cell-mediated cytotoxicity by KSHV K5 protein. In *3rd International Workshop on Kaposi's Sarcoma-associated Herpesvirus and Related Agents*, Amherst, MA, USA, 6–10 June 2000.
  129. Rimessi P, Bonaccorsi A, Fabris M, et al. HHV-8 ORF K3 is encoded by a bicistronic multisplliced immediate-early mRNA expressed upon virus reactivation. In *3rd International Workshop on Kaposi's Sarcoma-associated Herpesvirus and Related Agents*, Amherst, MA, USA, 6–10 June 2000.
  130. Stürzl M, Roth WK, Brockmeyer NH, Zietz C, Speiser B, Hofschneider PH. Expression of platelet-derived growth factor and its receptor in AIDS-related Kaposi's sarcoma in vivo suggests paracrine and autocrine mechanisms of tumor maintenance. *Proc Natl Acad Sci USA* 1992, **89**, 7046–7050.
  131. Sciacca FL, Stürzl M, Bussolino F, et al. Expression of adhesion molecules, platelet-activating factor, and chemokines by Kaposi's sarcoma cells. *J Immunol* 1994, **153**, 4816–4825.
  132. Bohan-Morris CB, Gendelman R, Marrogi AJ, et al. Immunohistochemical detection of bcl-2 in AIDS-associated and classical Kaposi's sarcoma. *Am J Pathol* 1996, **149**, 1871–1885.
  133. Saville MW, Lietzau J, Pluda JM, et al. Treatment of HIV-associated Kaposi's sarcoma with paclitaxel. *Lancet* 1995, **346**, 26–28.
  134. Sgadari C, Toschi E, Palladino C, et al. Mechanism of Paclitaxel activity in Kaposi's sarcoma. *J Immunol* 2000, **165**, 509–517.
  135. Rivas C, Thlick AE, Parravicini C, Moore PS, Chang Y. Kaposi's sarcoma-associated herpesvirus LANA2 is a B-cell-specific latent viral protein that inhibits p53. *J Virol* 2001, **75**, 429–438.
  136. Prakash O, Tang Z-Y, He Y, et al. Human Kaposi's sarcoma cell-mediated tumorigenesis in human immunodeficiency type 1 Tat-expressing transgenic mice. *J Natl Cancer Inst* 2000, **92**, 721–728.