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Kaposi's Sarcoma: A Result of the Interplay among Inflammatory Cytokines, Angiogenic Factors and Viral Agents

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Kaposi's sarcoma (KS) is an angioproliferative disease occurring in 4 clinic-epidemiologic forms. Although the AIDS-associated KS (AIDS-KS) is the most aggressive, all forms of KS share the same immunological and histopathological features suggesting common etiological and pathogenic factors. Recent data indicate that at least in early stage KS is not a real sarcoma but an angio-hyperplastic-inflammatory lesion mediated by inflammatory cytokines and angiogenic factors, that is triggered or amplified by infection with human herpesvirus-8. In addition, the human immunodeficiency virus type-1 Tat protein appears to be responsible for the higher grade of aggressiveness of AIDS-KS as compared to the other forms of KS. However, given time, reactive KS may progress to a sarcoma as suggested by evidence of monoclonality in late-nodular lesions. © 1998 Elsevier Science Ltd. All rights reserved.

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Kaposi's sarcoma (KS) is a multifocal proliferative disease of vascular origin found in 4 clinic-epidemiologic forms. AIDS-associated KS (AIDS-KS) is the most frequent tumor of human immunodeficiency virus type 1 (HIV-1) infected homo-bisexual men and is the most aggressive form of KS [1–3]; African KS (AKS) is frequent in certain areas of Africa where it can represent up to 10% of the total tumors and acquires a very aggressive course after HIV-1 infection [4, 5]; classical KS (CKS) occurs in elderly men of the Eastern-Mediterranean area and is a milder form of the disease [6, 7]; post-transplant KS (PKS) occurs in transplanted individuals after therapy with cyclosporin and corticosteroids [8, 9]. Although these forms have a different geographical distribution and clinical course they share many common features including (i) a disturbance of the immune system characterized initially by immunoactivation particularly of CD8 T cells with Th1-type cytokine production and later, at least for AIDS-KS and PKS, by immunosuppression; (ii) increased levels of circulating spindle cell progenitors; (iii) histopathology of the lesions; (iv) high levels of the same inflammatory cytokines (IC), angiogenic molecules and growth factors in the lesions; and (v) infection by human herpesvirus-8 (HHV-8). These and other features of KS lesions and KS patients suggest that the different epidemiological forms of KS are mediated by the cooperation of the same cytokines and viral agents.

In vitro and *in vivo* experimental data and clinical observations indicate that KS may not be a true sarcoma at least in early stages but it can develop as a reactive process mediated by IC and angiogenic factors whose production is triggered or enhanced by infection with HHV-8. In this context the Tat protein of HIV-1 can increase the frequency of development and the aggressiveness of AIDS-KS due to both its molecular mimicry of extracellular matrix (ECM) molecules that increases the effect of angiogenic factors and to the activation of IC production.

The role of cytokines, the lack of malignancy of isolated cell cultures, the lack of chromosomal alterations, the onset of KS as simultaneous multiple lesions in the absence of obvious metastasis and lastly, the sporadic

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cases of tumor regression support the hypothesis of the reactive nature of KS [10–13]. However, recent evidence also suggests that in later stages of development reactive KS lesions may transform to a true sarcoma.

PATHOLOGY OF KS LESIONS: NATURE OF THE INFLAMMATORY CELL INFILTRATE AND ORIGIN OF THE SPINDLE CELLS

KS lesions are characterized by multiple patch, plaque or nodular lesions particularly on the skin of the extremities but often involving also the mucosas and visceral organs, particularly in AIDS-KS. The nodular stage represents a late "tumoral" stage of the lesions and is often found at onset in AIDS-KS patients from Africa [14–16].

Histologically, early lesions are characterized by an inflammatory-granulation type reaction with activated proliferating endothelial cells which form new blood vessels often abnormal that allow extravasation of red blood cells and edema. This can precede the appearance of the typical "spindle cells" (KS cells (KSC)) that are considered to be the tumor cells of KS. On time, the spindle cells become the predominant cell type and the lesions acquire a more monomorphic aspect resembling a fibrosarcoma, although angiogenesis remains always a prominent feature [14, 17–22].

The nature of the inflammatory cell infiltrate of KS appears of importance since it is the first to appear and precedes the spindle cell formation. Immunohistochemical studies indicate a prevalent infiltration of T cells dominated by $CD8^+$ T cells but also containing $CD4^+$ T cells, numerous monocyte-macrophages ($CD4^+$, $CD14^+$, $CD68^+$, $CD45^+$, PAM-1^+) often with a spindle-like morphology and a subendothelial localization (Fig. 1), dendritic cells ($FXIIIa^+$) and few B cells ($CD19^+$, $CD20^+$ or $CD30^+$) [23–29]. The same features are also observed by analysing tumor infiltrating lymphocytes (TIL) and macrophagic spindle cell cultures derived from the lesions (Table 1) [30]. In addition, the enhanced expression of adhesion molecules in resident vessels and the lack of evidence of monocytic cell proliferation in KS indicate that monocytes are recruited from the blood and differentiate in loco in macrophages and dendritic cells [24]. As discussed later, these inflammatory cells, mostly $CD8^+$ T cells and monocytes-macrophages, produce a variety of IC and in particular γ-interferon (γIFN), that function in a synergistic fashion to activate endothelial cells, to induce the production of angiogenic factors and a further recruitment of T cells and monocytes.

The nature of the spindle cells of KS lesions has been debated for many years, however, recent data indicate that spindle cells are an heterogeneous cell population dominated by activated vascular endothelial cells (FVIII-RA$^+$, VE-Cadherin$^+$, PAL-E$^+$, ULEX$^+$, $CD34^+$, $CD36^+$, $CD31^+$, ICAM-1^+, V-CAM-1^+, ELAM-1^+, $CD40^+$, DR^+) mixed with macrophagic spindle-shaped cells ($CD14^+$, $CD68^+$, $CD31^+$, $CD36^+$, $CD4^+$, $CD45^+$, PAM-1^+, DR^+, ICAM-1^+) (Table 1 and Fig. 1) [23–25, 27, 28, 30–41]. FVIII-RA expression generally tends to decrease with lesion progression likely due to its release from the cells (see below).

Both endothelial spindle cells (E-KSC) and macrophagic spindle cells (M-KSC) have been established from the lesions and long-term cultured by utilizing the same IC expressed in the lesion but with modifications of γIFN and interleukin-2 (IL-2) content [30, 42, 43]. These cells possess the same phenotype as *in situ* KS spindle cells of both endothelial and macrophagic phenotype, respectively (Table 1) [23–25, 30, 34].

The vascular origin of most spindle cells is also suggested by experimental data, discussed below, indicating that IC increased in KS lesions are capable to induce normal endothelial cells to acquire the KS spindle cell phenotype [23, 36, 44–47]. This supports the concept of the reactive nature of these cells, at least in the earlier phases of lesion development.

The reactive or hyperplastic E-KSC are not transformed nor they induce tumors in nude or SCID mice, however, they promote highly angiogenic lesions of mouse cell origin that closely resemble early human KS lesions [48–51]. These lesions regress as early KS lesions can regress in humans [12] and as discussed below, are mediated by the angiogenic cytokines and growth factors produced by KS cells. However, although most spindle cells and, perhaps all in early stage, are reactive cells, recent evidence suggests that E-KSC are "transdifferentiated" cells (see below) and that in late stage they may transform.

Two transformed cell lines have been established from KS lesions that are able to give tumors in SCID but not in nude mice [52, 53] suggesting that tumorigenic growth may require a serious host immunodeficiency. In addition, recent studies on nodular AIDS-KS lesions from African women indicate monoclonality of spindle cells [54]. However, due to the mixed cellularity, this type of studies cannot be performed on early lesions. On the other hand, others have also found polyclonality of the lesions [55], suggesting that tumor transformation may occur in some cases of advanced KS patients that are severely immuno compromised such as African AIDS-KS patients. Consistent with this, microsatellite instability has been observed in AIDS-KS but not in the absence of HIV-1 infection such as in CKS lesions [56].

IMMUNOACTIVATION IN KS PATIENTS AND IN INDIVIDUALS AT HIGH RISK OF KS: CD8 T-CELL ACTIVATION AND Th-1 CYTOKINE PROFILE

All patients with KS or at high risk of KS have signs of immunoactivation and KS itself can arise in the absence of immunodeficiency [57]. For example, homosexual men have increased blood levels of ICAM-1, soluble CD8, neoprotein levels and other signs of activation even prior to HIV-1 infection or after HIV-1 infection but prior to KS development [36, 58–70]. In these individuals KS can arise prior to HIV-1 infection but in a

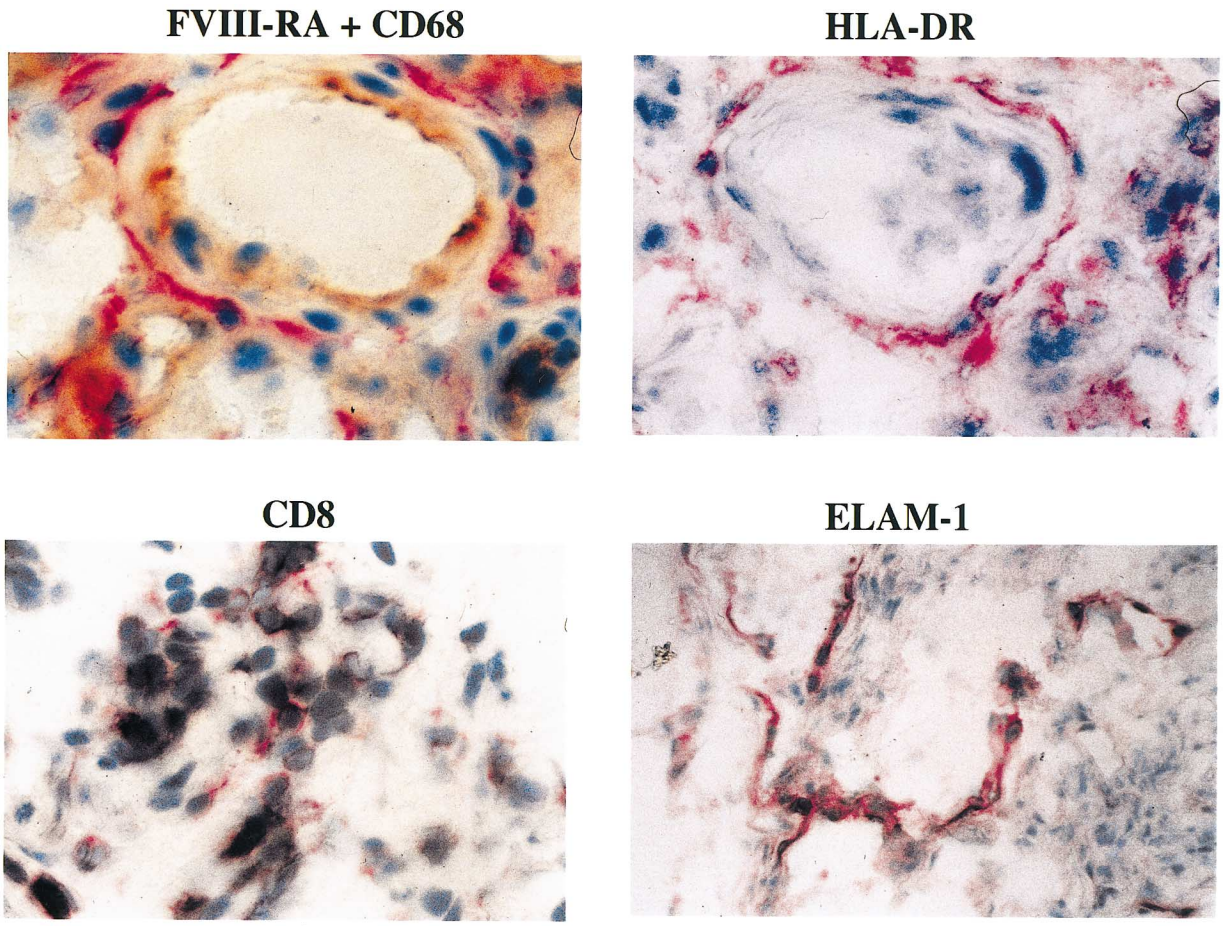

Figure 1. Detection of macrophages ($CD68^+$), CD8 T cells and activation markers (HLA-DR and ELAM-1) in KS lesions. Single and double immunostaining experiments with specific antibodies were performed as described [23, 50]. FVIII-RA staining is in brown and CD68 staining in red. A prominent infiltration by $CD8^+$ cells and $CD68^+$ (also $CD14^+$) monocytes-macrophages with a spindle-like morphology and a subendothelial localization is detected in all forms of KS. This is associated with the expression of activation markers including HLA-DR and ELAM-1 expression in both vessels and spindle cells, as compared to uninvolved tissues [23, 34].

milder form. Similarly, very recent and yet unpublished data indicate that KS can arise in HIV-1 infected homosexual men that are long-term non progressors (Ensoli B., unpublished data). These patients are generally characterized by a very low HIV-1 viral load, lack of immunodeficiency, CD8 activation and anti-HIV viral activity. Again these forms of KS appear to be mild and localized. African individuals are also immunoactivated probably due to frequent infections of different types [71–73]. Elderly men can present an oligoclonal CD8 expansion with increased production of IL-1 and tumor necrosis factor α (TNFα) and have no signs of immunosuppression at onset of KS [74–76]. Post-transplanted individuals receive large quantities of alloantigens which may lead to local foci of immunostimulated cells even under conditions of clinically induced immunosuppression.

These and other clinical observations suggest a role for a CD8 T-cell activation and production of IC of the Th-1 type (γIFN and IL-2) in KS development. Recent evidence indicates that this is the case. In fact, activated peripheral *blood* mononuclear cells (PBMC) from both AIDS-KS and CKS patients produce high levels of γIFN and little or no IL-4 as compared to patients without KS but with other dermatological disorders [30]. CD8 T-cell activation and infiltration and production of IC by CD8 T cells and monocytes-macrophages is also found in KS lesions from the same patients (Fig. 1 and see below) [30, 23]. Thus, immunoactivation is a trait of individuals developing KS and production of IC including γIFN, IL-1, TNFα appears to be key to KS development. In fact, the administration of γIFN, IL-2 or TNFα to KS patients leads to disease progression or to KS development [77–80]. Disease progression is also observed during opportunistic infections [81] that are naturally associated with IC production.

A systemic increase of IC may be responsible for several features of KS patients (Table 2) such as (i) the presence of circulating spindle cell precursors (see below); (ii) activation of vessels (Fig.1) and increased circulating levels of FVIII-RA [82, 83], an indicator of endothelial cell activation and damage [36, 84], and (iii) increased vascular adhesiveness with extravasation and tissue recruitment of lymphocytes and monocytes, as suggested by recent studies with HIV-1 infected individuals [24, 85].

Table 1. *In situ* KS spindle cells and spindle cells cultured from lesions or from blood are of endothelial or macrophagic cell origin and express activation molecules

Marker	Specificity	Cultured E-KSC	*In situ* E-KSC	Cultured M-KSC	*In situ* M-KSC	Cultured circulating KSC progenitors
FVIII-RA	Vascular endothelium	±[a]	±[b]	−	−	−[c]
CD34	Vascular endothelium and hematopoietic cell progenitors	+	+	−	−	−[c]
VE-Cadherin	Vascular endothelium, endothelial macrophages	+	+	n.d.	n.d.	+
CD31	Macrophages, endothelial cells	+	+	+	+	+
CD14	Monocytes-macrophages	−	−	+	+	+
CD68	Tissue macrophages	−	−	+	+	+
CD36	Macrophage, capillary endothelium	+	+	n.d.	+	n.d.
PAM-1	Macrophages	−	−	+	+	+
CD45	Leukocytes	−	−	+	+	+
CD4	T cells, monocytes-macrophages	−	−	+	+	+[d]
HLA-DR	Macrophages, activated endothelial cells, others	+	+	+	+	+
ICAM-1	Macrophages, activated endothelial cells, others	+	+	+	+	+
VCAM-1	Activated endothelial cells, others	+	+	n.d.	n.d.	n.d.
ELAM-1	Activated endothelial cells	+	+	−	−	n.d.
CD40	Vascular endothelium	+	+	n.d.	n.d.	n.d.
$\alpha 5\beta 1$ and $\alpha v\beta 3$	Activated endothelial cells, others	+	+	n.d.	n.d.	n.d.

E-KSC and M-KSC, endothelial- or macrophagic-KS spindle cells, respectively. (+), positive expression; (−), negative expression; n.d., not done.
[a] Positive after culture in the absence of TCM.
[b] Positive in early stage KS, lost in late stages.
[c] In a previous report few weakly positive cells have been detected [86].
[d] No expression has been detected in 1 report [86].
Endothelial and macrophagic KS spindle cells derived from lesions, spindle cells derived from blood after 6–7 days of culture and frozen sections of AIDS-KS and CKS lesions were stained by immunohistochemistry for the indicated markers [50, 23, 24, 25, 30, 36, 41, 88]. All cells were grown with IC with modification in some cytokine content, as described in the text and elsewhere [23, 30, 36].

Finally, in AIDS-KS patients, IC activate also HIV-1 replication and further production of the viral Tat protein that acts as a progression factor in AIDS-KS (see below).

CIRCULATING SPINDLE CELL PROGENITORS: A TRAIT OF KS PATIENTS

Circulating spindle cell progenitors have been found in patients with all forms of KS and in individuals at high risk to develop KS such as HIV-1 infected homosexual men [86, 87]. In KS patients these cells arise spontaneously in the adherent cell fraction of cultured PBMC. After short-term culture most of the adherent cells from these patients acquire a spindle morphology, others acquire a typical macrophagic morphology and all express markers of tissue macrophages including CD14, CD68, PAM-1, CD4, CD45, CD31 (Table 1) [86–88]. In addition, a proportion of these cells acquires expression of VE-cadherin, a marker of vascular endothelial cells, although they remain negative for FVIII-RA and CD34 [87, 88] (Table 1). This phenotype resembles an unusual cell type found in lymph nodes, the so called endothelial macrophages [89].

Although at a lower number, these spindle cells can also be obtained from high risk individuals and at a much lower prevalence, from normal blood donors, however, this requires the addition to the PBMC of the same IC increased in KS patients [86] (Ensoli B., unpublished data). This suggests that IC production in KS is responsible for inducing an expansion of this cell type. A greater number of these cells is also found in KS patients as compared to matched patients but without KS (i.e. AIDS-KS homosexual patients vs AIDS homosexual patients) [86, 88]. These cells disappear after effective therapy [87] suggesting that besides its role in disease pathogenesis, this cell type may represent a prognostic marker in KS patients.

The presence of these cells in the blood may suggest an explanation for the multifocal lesions developing in KS patients. In fact, they resemble very closely the phenotype of the M-KSC [30], and, as discussed below, in KS patients they are infected by HHV-8 suggesting that they can carry the virus to tissues and differentiate in loco into

Table 2. Systemic and tissue (KS) localized effects of combined IC in KS pathogenesis

Systemic effects
Expansion of circulating spindle cell progenitors and differentiation to macrophages and endothelial nacrophages.
Vessel activation (increased FVIII-RA serum levels) and extravasation of inflammatory cells.
Activation of HIV-1 gene expression/replication and production/release of HIV-1 Tat protein.
Activation of HHV-8 infection and increase of viral load.
Local (KS) effects
Inflammatory cell recruitment, differentiation (monocytes to macrophages and dendritic cells) and survival (maintain survival and phenotype of TIL from KS lesions).
Growth induction and establishment of E-KSC and M-KSC from lesions.
Enhancement of cytokine production and *in vivo* angiogenic activity of E-KSC (bFGF, VEGF, GM-CSF, IL-6, IL-8, IL-1, MCP-1 etc.).
Endothelial cell activation and induction of the same features of E-KSC:
Spindle cell morphology.
Downregulation of FVIII-RA due to release.
Upregulation of adhesion molecules and activation marker expression (ICAM-1, VCAM-1, ELAM-1, HLA-DR, CD40, $\alpha5\beta1$, $\alpha v\beta3$).
Induction of bFGF, IL-6, IL-8, MCP-1, GMCSF, IL-1, PDGF-A production. Activation of bFGF release.
Induction of the responsiveness to the proliferative, chemotactic, invasive and adhesive effects of HIV-1 Tat protein (induction of $\alpha v\beta3/\alpha5\beta1$ and bFGF that are required for Tat activity).
In vivo angiogenic and KS-like forming activity in synergism with the HIV-1 Tat protein.
Induction of KS-like lesions after injection in nude mice that are increased synergistically by HIV-1 Tat protein.

Among the IC expressed in KS (see Table 3), γIFN appears to be the most expressed and a key (necessary) factor to induce all the effects described above. However, IC combined (particularly γIFN, TNF, IL-1) have synergistic effect in inducing both systemic and local effects (see text for details).

macrophages and endothelial macrophages as occurs *in vitro* [88].

INFLAMMATORY CYTOKINES ARE EXPRESSED IN KS LESIONS AND TRIGGER KS LESION FORMATION

A variety of IC are expressed in lesions from all forms of KS. These include γIFN, TNF, IL-1, IL-6, granulocyte/macrophages colony stimulating factor (GMCSF), and others [23, 30, 90–92] (Tables 2 and 3 and Fig. 2). They are all produced by infiltrating lymphocytes and monocytes-macrophages. In addition, IL-1, IL-6 and GMCSF are also produced by activated endothelial cells and E-KSC [49, 90, 92–94]. This IC production is associated with vessel activation (ICAM-1$^+$, ELAM-1$^+$, V-CAM-1$^+$, DR$^+$, CD40$^+$, upregulation of $\alpha5\beta1$ and $\alpha v\beta3$ integrins) (Figs 1 and 2) [23–25, 34, 50] and increased vascular adhesion of inflammatory cells [85] (Table 2). The same IC are produced by activated PBMC [43] and this mixture (T-cell activation conditioned media (TCM)) or recombinant cytokines added together at the same concentration as found in TCM, mediate, in a synergistic fashion, phenomena that appear to be key to KS lesion formation and progression (Tables 2 and 3).

TCM or combined IC induce the long-term growth of cultivated E-KSC, and in fact, they have been previously used to establish E-KSC from the lesions [42, 48, 49]. Several of the IC present in TCM (IL-1 α and β, TNFα and β, γIFN, Oncostatin M (OSM)) contribute to induce the long-term growth of hyperplastic E-KSC [42, 43, 46, 49, 95]. Oncostatin M, in particular, has been found to be a strong KS cell growth factor for some E-KSC [96–99], likely via induction of basic fibroblast growth factor (bFGF) [100] but has inhibitory growth effects on other KSC cultures [92]. These controversial results have also been reported for the activity of OSM on endothelial cells [100, 101] and it may be related to different cell culture conditions or to the preparation of OSM used in these studies. However, it is clear that the effect of IC on KSC growth is mediated by a synergistic stimulatory effect on bFGF production and release. bFGF, in turn, functions as an autocrine KS cell growth factor [35, 46, 49–51, 95]. IC also increase the *in vivo* angiogenic and KS-forming activity of KS spindle cells [46], suggesting that IC can maintain and enhance KS growth and progression (Tables 2 and 3).

IC also support the establishment of M-KSC from the lesions (Table 2). As mentioned above, this can be obtained by modifying the concentrations of the different cytokines present in the IC mixture [30]. This confirms immunohistological studies showing that both endothelial and macrophagic spindle cells are present in the lesions (Table 1). However, these cell types require slight modifications in culture conditions for growth (Table 2) [30]. It cannot be excluded that a pluripotent precursor cell may differentiate into both cell types. In this case, the circulating spindle cell progenitors are the best candidate for this role [86, 87].

IC are also able to maintain in culture KS-derived TIL with the same phenotype as those found *in situ* in KS lesions (Table 2), whereas, in the absence of TCM, these cells undergo apoptosis and disappear rapidly [30]. Thus, besides the activation of endothelial cells which seems to be important for KS initiation, IC can also maintain KS lesions by promoting cell survival and growth.

The same IC activate endothelial cells to acquire the phenotypic and functional features of E-KSC (Tables 1, 2 and 3) [23, 36, 43–45, 47]. These include a typical spindle morphology and the expression of the same markers (downregulation of FVIII-RA, activation of ELAM-1, ICAM-1, V-CAM-1, DR, $\alpha5\beta1$, $\alpha v\beta3$ integrin expression) [36]. In particular, FVIII-RA positivity tends to be lost by *in situ* E-KSC of progressive KS lesions or by culturing E-KSC or endothelial cells in TCM due to its release that is induced by IC, but FVIII-RA positivity is regained by omitting TCM from the cultures [36, 47]. This suggests

Table 3 Expression and activity of inflammatory cytokines in KS

Factor	Expression in KSC *in vitro*	Activity on KSC *in vitro*	Expression in KS *in vivo*	Expression of cognate receptors in KSC *in vivo*	Possible role in KS pathogenesis
IL-1α*	+	+ (M,P)	−	n.d.	Activation of E-KSC proliferation mediated by bFGF, activation of endothelial cells, cell recruitment.
IL-1β*	+	+ (M,P)	+	n.d.	Activation of E-KSC proliferation mediated by bFGF, activation of endothelial cells, cell recruitment.
IL-6*	+	? (P)	+	gp130 (+), IL-6R (−)	Lack of IL-6R expression in KS lesions *in vivo* argues against a role of IL-6. Biological activity on KSC may be mediated by circulating soluble IL-6R.
TNFα,β*	−	+ (M,P)	+	n.d.	Activation of E-KSC proliferation, activation of endothelial cells, cell recruitment. KS progression after systemic inoculation.
IFNγ*	−	+ (M,P)	+	n.d.	Activation of endothelial cells (EC), induce phenotypic transformation of EC to E-KSC, cell recruitment. KS progression after systemic inoculation.
OSM	?	? (P)	?	gp130 (+), LIF-R (−)	It is still in discussion whether OSM is an activator or an inhibitor of E-KSC proliferation *in vivo* and *in vitro*.

KSC, KS spindle cells; n.d., not done; (+) and (−), expression or activity present absent, respectively; (*), increased serum concentrations in KS patients. (?), conflicting data; (P), proliferation, (M), morphologic transformation (spindle shape). gp130 (+), expression of the signal transducing molecule gp130. IL-6R (−), LIF-R (−), lack of expression of the binding molecules which constitute together with gp130 the high affinity receptors for IL-6 and OSM, respectively.
IC are mostly produced by infiltrating inflammatory cells (CD8 T cells and monocytes-macrophages) and some (IL-1, IL-6) by spindle cells. IC are also increased in high risk patients and KS patients and act synergistically to induce both systemic and tissue-localized effects (Table 2).

that IC may contribute to the increased blood levels of FVIII-RA found in HIV-1 infected individuals.

IC produced in KS lesions also induce cultured endothelial cells and E-KSC to produce angiogenic factors (Tables 2 and 3) such as bFGF, vascular endothelial cell growth factor (VEGF), IL-8, platelet derived growth factor (PDGF-A) and other cytokines and chemokines expressed in primary lesions with effects on cell recruitment, growth, angiogenesis and lesion formation (discussed below). In addition, upon exposure to IC endothelial cells become angiogenic in nude mice and induce formation of KS-like lesions as E-KSC do [23, 36, 47]. Similarly, inoculation of IC induces KS-like angiogenic lesions in mice [102], indicating that they can trigger a cascade of events leading to lesion formation (Table 2).

IC also induce normal endothelial cells to become responsive to the adhesive, mitogenic and invasive effects of extracellular HIV-1 Tat protein as E-KSC (Table 2) and as discussed below, this is due to both activation of the expression of the receptors for Tat ($\alpha5\beta1$ and $\alpha v\beta3$ integrins) and induction of bFGF production that are constitutively expressed by established E-KSC. This leads to augmented angiogenesis and spindle cell growth in AIDS-KS [50].

γIFN appears to be the major mediator of these changes although the other IC, particularly IL-1 and TNF, contribute to these effects in a synergistic fashion (Fig. 2 and Tables 2 and 3) [23, 36, 46, 47, 103]. In addition, γIFN upregulates CD40 expression in cultured E-KSC [41]. CD40 is also highly expressed by KS spindle cells of AIDS-KS and CKS lesions and by vascular endothelial cells in areas within and adjacent to the tumors [41]. This and the HLA-DR expression in KS lesions (Fig. 1) indicate that γIFN is active on KS spindle cells and endothelial cells in KS lesions *in vivo*. Signaling through CD40 is able to prevent apoptosis, probably by induction of the expression of the bcl-2 proto-oncogene [104]. This suggests that γIFN-induced expression of CD40 in KS spindle cells and endothelial cells seen *in vivo* may contribute to the increase of bcl-2 expression that is observed in the same cells during progression of all forms of KS [105] (see below).

Although IC activate endothelial cells to acquire the E-KSC phenotype a few differences still exist as compared to E-KSC. These include the lack of production of VEGF [106] and the lack of a growth response to RGD peptides [102]. E-KSC, in fact, produce VEGF and this expression is increased by IC [106–108]. In addition, E-KSC proliferate with RGD peptides suggesting alterations in the integrin pathway [102]. In contrast, IC-activated endothelial cells do not. Finally, IC-activated endothelial cells but not E-KSC proliferate in response to VEGF although both express similar receptors levels [106, 108]. This suggests that E-KSC have acquired a "transdifferentiated" phenotype although they are not transformed nor tumorigenic in SCID mice.

Finally, IC activate HIV-1 transcription, replication and production of Tat in infected cells [109] and, as suggested by recent studies discussed below, they activate HHV-8 replication and increase viral load (Table 2).

Altogether these results indicate that the IC produced

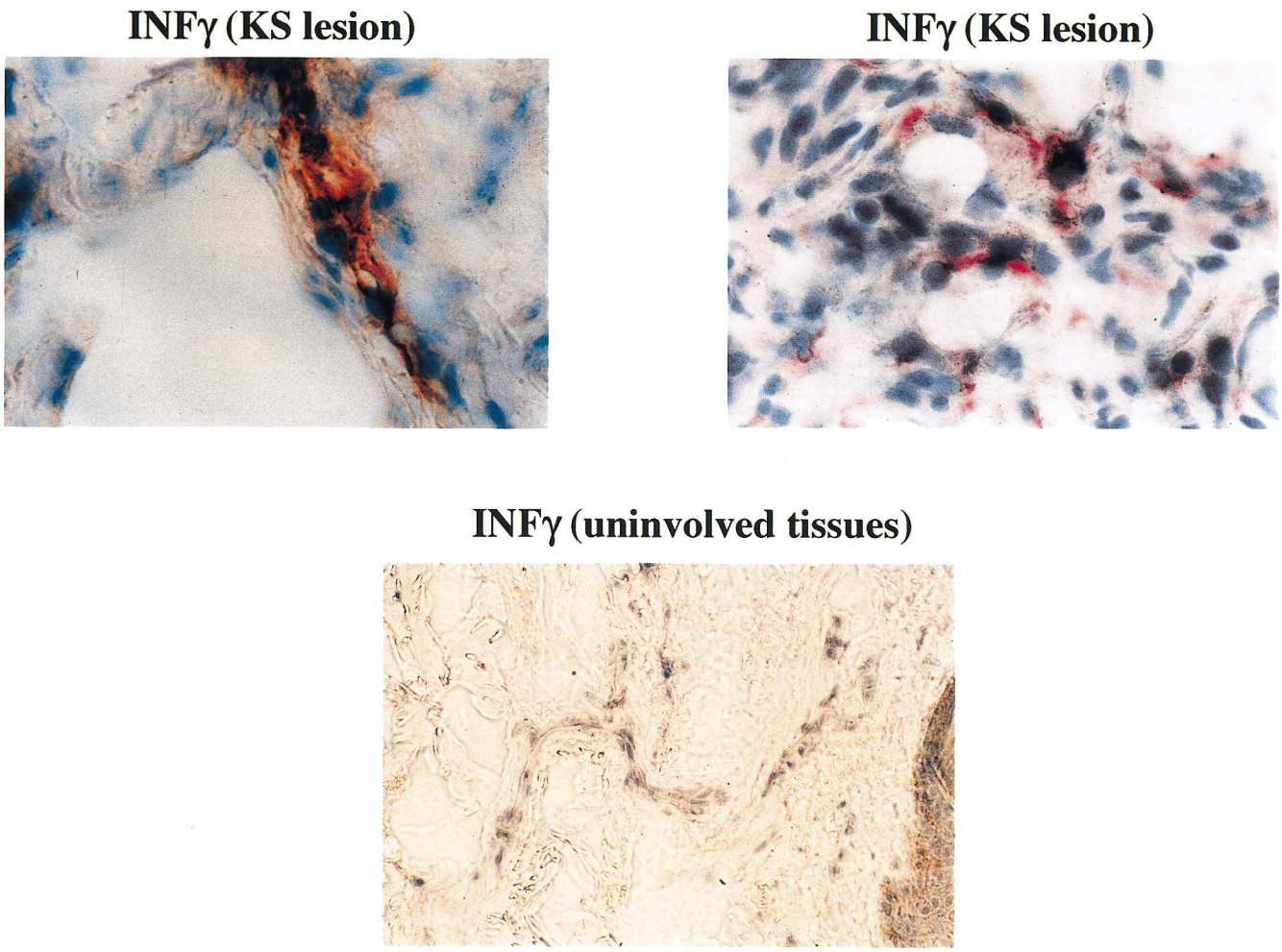

Figure 2. Immunohistochemical detection of γIFN expression in two different KS lesions (upper panel) as compared to uninvolved tissues (lower panel). γIFN is produced by both $CD8^+$ T cells and monocytes-macrophages with spindle morphology, as determined by immunohistochemistry with specific antibodies and by double staining experiments with antibodies specific for γIFN and CD8 T cells or monocytes-macrophages (CD68 and CD14) [23].

in KS lesions are capable of triggering a cascade of events leading to lesion formation and to maintenance and progression of KS.

ANGIOGENIC MOLECULES, GROWTH FACTORS AND CHEMOKINES MEDIATE KS LESION FORMATION

Angiogenesis, proliferating spindle cells and prominent infiltration of inflammatory cells are the characteristic histological features of KS. Several different angiogenic molecules, growth factors, and chemokines appear to mediate these phenomena.

The first experimental evidence that angiogenic factors are involved in KS lesion formation was provided by studies indicating the capability of E-KSC to induce angiogenesis in the chorioallantoic membrane assay and highly angiogenic KS-like lesions after inoculation of the cells in nude mice [48–51]. These KS-like lesions are of mouse cell origin, regress in time and are mediated by specific angiogenic factors produced by the cells. In particular, bFGF is a key mediator of lesion formation (Tables 2 and 4).

Inoculation of bFGF in nude mice results in the formation of KS-like lesions [50]. bFGF is expressed at very high levels by E-KSC *in vitro* and *in vivo* [49–51] and it is released by these cells in the absence of cell death or cell permeability changes [46, 47, 49]. Finally, inhibition studies with specific neutralizing antibodies or antisense oligodeoxynucleotides directed against bFGF mRNA have shown that bFGF is required for the formation of KS-like lesions induced by inoculation of E-KSC in nude mice [51].

In addition to its paracrine activity, bFGF has autocrine activity in KS development because it stimulates proliferation of E-KSC and IC-activated endothelial cells [46, 47, 51, 95, 99]. Most importantly, both bFGF mRNA [110] and protein [50] are highly increased in tissue sections of KS primary lesions and in KS-like mice lesions [36, 46, 47], which indicates that bFGF regulates angiogenesis and E-KSC growth in both humans and mice.

As mentioned above IC induce, in a synergistic fashion,

bFGF production and release in both E-KSC and normal endothelial cells [23, 46, 47, 95, 99], and IC-activated endothelial cells induce KS-like lesions in nude mice that are mediated by bFGF and are indistinguishable from those induced by E-KSC (Table 2) [23, 36, 47]. γIFN is essential also for this effect although TNF and IL-1 contribute and synergize with γIFN to induce angiogenic activity of the cells [23, 47].

However, neutralizing anti-bFGF antibodies or antisense oligodeoxynucleotides do not totally block lesion formation after inoculation of E-KSC in mice [51], and injection of bFGF alone does not induce the edema characteristic of KS [50]. This suggests involvement of other factors. In fact, VEGF, another angiogenic factor, is expressed as the two secreted forms (VEGF 121, VEGF 165) in both KS lesions and in cultured E-KSC [106, 108] (Fig. 3 and Table 4). As for bFGF, VEGF expression in E-KSC is also induced by IC and by other cytokines found in KS lesions such as PDGF-B [106, 108]. VEGF synergizes with bFGF in inducing endothelial cell growth and angiogenesis as demonstrated by *in vitro* and mice studies (Table 4) [106, 108]. In addition, bFGF and VEGF synergize to induce edema as shown by injecting both cytokines alone and combined in guinea pigs [106]. VEGF does not induce the growth of E-KSC described above although the cells express both its receptors (KDR/FLK-1 and flt-1) [106, 108] but appears to mediate the growth of the two transformed KS cell lines established from KS lesions [111].

Expression of KDR/FLK-1 receptor tyrosine kinase, one subunit of the VEGF receptor, has also been detected in KS spindle cells *in vivo* [112], suggesting that VEGF may have some autocrine activity on KS spindle cell proliferation in progressed stages of the disease. Nevertheless, since KS spindle cells were identified only by morphologic criteria [112], it is unclear whether VEGF and FLK/KDR-1 are expressed by the same or different spindle cell populations (E-KSC or M-KSC). However, VEGF clearly contributes to the angiogenesis and edema characteristic of KS.

Another angiogenic molecule found in KS is the scatter factor/hepatocyte growth factor (SF/HGF) (Table 4) [113, 114]. SF/HGF induces endothelial cells to acquire a spindle morphology and stimulates proliferation of cultured KS spindle cells [114]. Moreover, SF/HGF and its cognate receptor, the c-*met* protein, are expressed in human KS lesions [114, 115], suggesting that it may play a role in KS development.

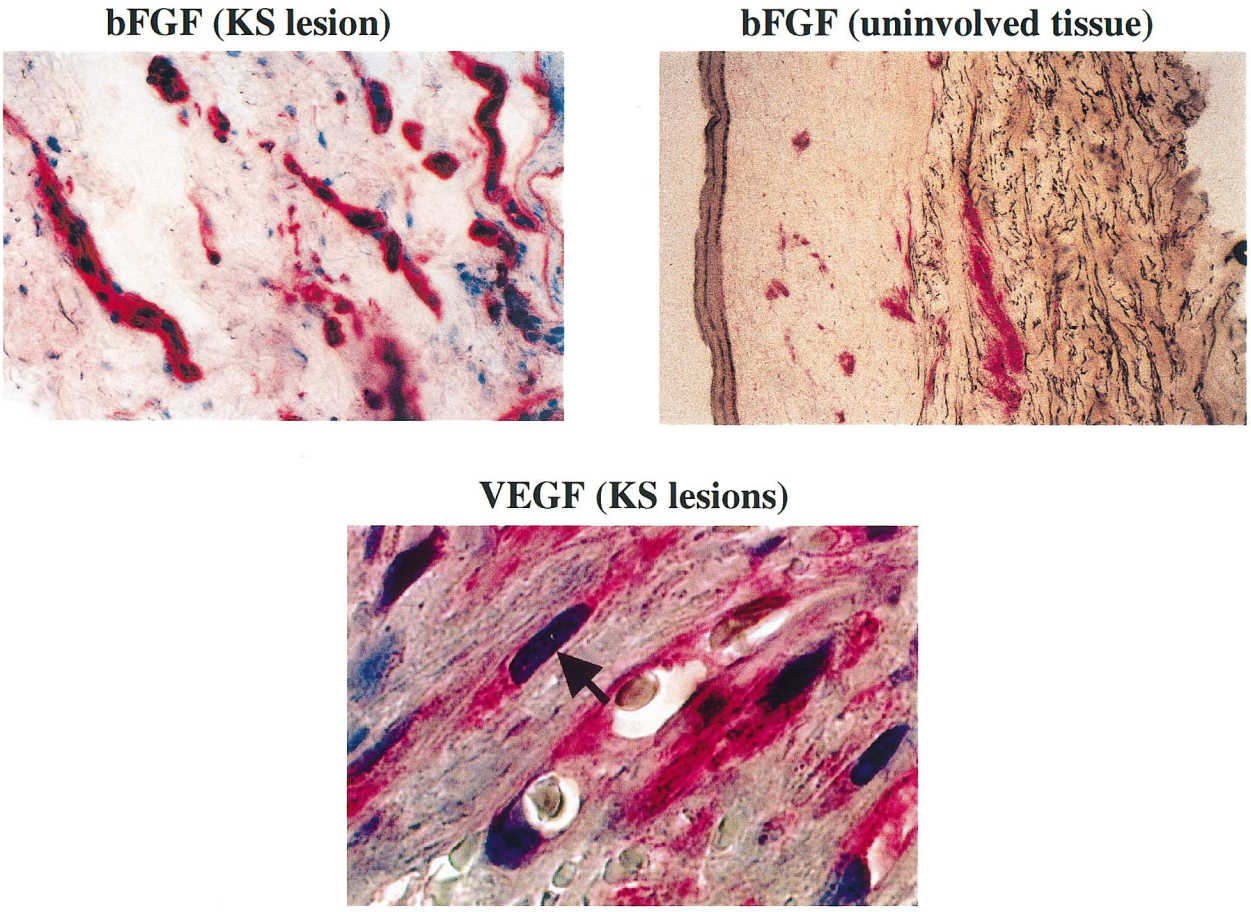

Figure 3. Expression of bFGF and VEGF in KS lesions as compared to uninvolved tissues. Both angiogenic factors are expressed at high levels in all forms of KS. Generally, the number of bFGF positive cells is higher (about double) than that of VEGF. Expression of both factors in spindle cells was determined by immunohistochemistry [23, 50, 108, reproduced with permission of the American Journal of Pathology].

Table 4. Expression and activity of growth and angiogenic factors in KS

Factor	Expression in KSC *in vitro*	Activity on KSC *in vitro*	Expression *in vivo*	Expression of cognate receptors in KSC *in vivo*	Possible role in KS pathogenesis
aFGF	+	n.d.	+	+	May contribute to KS cell growth and angiogenesis (m.d.r.).
bFGF	+	+ (P,CH,I)	+	+.	Key role in E-KSC proliferation and angiogenesis. Acts in synergy with HIV-1 Tat protein and VEGF in induction of angiogenesis and edema.
FGF 3	n.d.	n.d	+	n.d	(m.d.r.)
FGF 5	+	n.d.	+	n.d.	(m.d.r.)
FGF 6	−	n.d.	+	n.d.	(m.d.r.)
VEGF	+	? (P)	+	+.	Contributes to KS associated edema and angiogenesis. Acts in synergy with bFGF.
PDGF-A	+	+ (P)	+	−	Activation of KSC proliferation, angiogenesis, absence of receptor expression *in vivo* argues against a role in KS development.
PDGF-B	?	+ (P, CH)	+	+	Role in KSC proliferation.
SF/HGF	+	+ (P)	+	+	May activate KSC proliferation and contribute to angiogenesis.
MCP-1	+	n.d.	+	n.d..	Chemoattractive for monocytes, may contribute to the high numbers of monocytes generally observed in KS lesions.
IL-8	+	+ (CH)	+	n.d.	May induce chemotaxis of endothelial cells, KSC and other cells in KS lesions.
GM-CSF	+	± (P)	+	n.d.	Monocyte differentiation to macrophages and dendritic cells.
PAF	+	+ (P, CH)	n.d.	n.d.	May contribute to angiogenesis (m.d.r.).
TGF-α	±	+ (P)	n.d.	n.d.	KSC proliferation (m.d.r.).
TGF-β	+	+ (P)	+	n.d.	KSC proliferation (m.d.r.).

KSC, KS spindle cells; n.d., not done; (+), (±) and (−) expression or activity present, weak or absent, respectively; (?), data are conflicting; (P), proliferation; (CH), chemotaxis; (I), invasion.
(m.d.r.), more data are required to evaluate the role of the respective factor in KS pathogenesis.

PDGF-B is another potent paracrine-acting mitogen for cultured E-KSC [116, 117] that is expressed *in vivo* by subpopulations of cells that are intermingled with the spindle cells [92, 118] (Table 4). E-KSC express PDGF β-receptor [92, 118], suggesting that PDGF-B may activate the proliferation of KS spindle cells by paracrine mechanisms. In addition, PDGF-B may have angiogenic activity suggesting that it may also contribute to the angiogenesis found in KS [119].

IL-1 is also produced and released by E-KSC [49] and expressed in human KS lesions (Table 3) [92]. IL-1 induces autocrine growth of E-KSC [49]. Recent studies, however, indicate that its growth effects are mediated by induction of bFGF production which appears to be the final mediator of KS cell growth [46, 47, 51, 95]. In addition, IL-1 synergizes with TNF and γIFN to induce endothelial cell activation and the acquisition of the KS cell phenotype [23, 36], and promotes leukocyte recruitment (Tables 2 and 3) [120].

IL-6 is produced by inflammatory cells, E-KSC and by IC-activated endothelial cells and although it has been shown to induce KS cell growth (Table 3) [90, 93], other reports have not confirmed these findings. In fact, E-KSC lack both *in vitro* and *in vivo* the IL-6 receptor [92, 121]. In addition, IL-6 does not induce endothelial cell growth and has no angiogenic activity in the nude mice model [120] (Ensoli B., unpublished data). However, *in vivo* biological activity of IL-6 on KS spindle cells may be mediated by circulating soluble IL-6 receptor molecules that may bind to the signal transducing molecule gp130 which is highly expressed by E-KSC *in vivo* [92, 122]. In addition, IL-6 can amplify leukocyte recruitment [120].

The prominent leukocyte infiltration present in KS has suggested that chemokines may be involved in KS development. In fact, IC induce the expression of monocyte chemotactic protein-1 (MCP-1) in E-KSC (Table 4) [120, 123]. Furthermore, MCP-1 has been detected in the spindle cells of KS lesions suggesting that MCP-1 may contribute to the recruitment of monocytes into KS lesions [123, 124].

IL-8 is another chemokine expressed in KS lesions, by cultured E-KSC and by IC-activated endothelial cells (Table 4) [123]. IL-8 has chemotactic activity for all immune cells identified so far. In addition, this cytokine may have a prominent role in endothelial cell migration since it appears to have more migratory than proliferating effects on E-KSC and endothelial cells (Ensoli B., unpublished data). However, by this effect IL-8 may also contribute to the angiogenesis found in KS lesions.

GMCSF is produced by E-KSC [49] and by infiltrating inflammatory cells of the lesions (Table 4). Although GMCSF may induce some angiogenic activity [125] it is

a weak inducer of KS spindle cell growth [43]. However, it may contribute to the differentiation of monocytes into macrophages and dendritic cells that are found in KS lesions [126].

Although other cytokines are expressed by spindle cells of KS lesions or by cultured E-KSC including acid FGF (aFGF) [39, 49, 127, 128], FGF-6 [128, 129], platelet activating factor (PAF) [130], PDGF-A [92, 116, 117], TGF-α [14, 131], and TGF-β [49, 115, 132, 133] (Table 4), their role in KS lesion formation is yet to be determined and will not be discussed further.

Altogether these data indicate that a network of angiogenic factors (bFGF, VEGF, SF/HGF, PDGF-B), spindle cell growth factors (bFGF, PDGF-B, IL-1), and chemotactic factors (MCP-1, IL-8) are expressed in KS and regulate recruitment, survival, growth and differentiation of the different cell types, including spindle cells, present in KS lesions. The biological activities of these molecules and of the IC discussed above can explain the mixed cellularity, the angiogenesis and the edema of KS lesions in the context of a cytokine-mediated reactive process.

HHV-8 A NEW HERPESVIRUS ASSOCIATED WITH KS: A TRIGGERING EVENT OR A CONSEQUENCE OF LESION FORMATION?

Although a transmissible agent has been postulated as the causal agent of KS and several viruses and other agents have been suggested [21, 22], none has been confirmed. Recently a new herpesvirus termed HHV-8, that is closely related to Epstein–Barr-virus (EBV) and herpesvirus saimiri, has been identified and shown to be present in all epidemiological forms of KS [134–140]. HHV-8 has also been found in primary effusion B-cell lymphomas, Castelman disease and in the dendritic cells of the bone marrow of patients with multiple myeloma [141–145]. Recent epidemiological studies by PCR on PBMC and by a first generation serogical assays indicate that HHV-8 is particularly prevalent in those geographical areas, including certain areas of Africa, Greece and Italy, with a high incidence of KS [146–149]. In these areas and, less frequently, in other areas of the world at a lower HHV-8 prevalence, the virus is also present in normal blood donors or in patients without KS [150–153]. However, in these individuals viral load in PBMC and tissues appear to be much lower than in patients with KS [154–157] and antibodies directed against viral latent antigens are less prevalent than in KS patients [147, 157, 158].

HHV-8 load in PBMC is also higher in HIV-infected individuals [159] and in Africans [160, 161] as compared to other groups at risk of KS. Similarly, a positive serology is found more often in homosexual men, in Africans and in elderly men of high risk geographical areas [146, 157]. Since HHV-8 seroprevalence is low in areas at low incidence of KS and its detection can precede the onset of KS [157, 162, 163], these results suggest that HHV-8 is key to KS development but it requires additional factors to exert its effects in KS pathogenesis.

In PBMC, the virus is detected in B cells [135, 150, 164, 165], but recent data indicate that it is also present in monocytes-macrophages [30, 166], dendritic cells [145] and more rarely (in advanced KS), in T cells [88]. Interestingly, HHV-8 is detected in the circulating monocytes and spindle cell progenitors of KS patients [87, 88], suggesting that these cells may play a role in virus recruitment into tissues.

At the lesion level, HHV-8 is present in endothelial and spindle cells mostly in a latent form, [167–171] whereas mononuclear cells including monocytes-macrophages are lytically infected (Fig. 4) [166, 172] and may support virus production and spread to other cell types. This is suggested by recent studies of HHV-8 transmission to PBMC, B cells, monocytes-macrophages, dendritic cells and endothelial cells (Goletti D., in preparation), and by *in situ* hybridization results showing the recruitment of HHV-8 infected monocytes into KS tissues (Fig. 4) [166]. In fact, although circulating B cells are infected and may represent one of the major reservoir of the virus, they are few or absent in KS lesions, whereas monocytes and T cells are much more abundant (Fig. 1). In addition, the virus is lost after culture of E-KSC from the lesions [173, 174], but it is maintained in the M-KSC cultures derived from the lesions [30].

The question whether extravasation of HHV-8 infected mononuclear cells into the tissue may be the initiating event of KS development or whether these cells are recruited secondarily into an early reactive focus of KS has not yet been solved. The second hypothesis, however, is supported by recent data showing that in late stage KS lesions numerous KS spindle cells express the latency associated nuclear antigen (LANA) and the kaposin gene (expressed in lytic and latent infection) of HHV-8 [167, 171], whereas in early KS lesions LANA expression is not detected, and the relative number of cells (i.e. the number of positive cells/total number of KS cells) expressing kaposin is much lower as compared to late stage lesions [167, 171]. Further, viral load increases with lesion stage and although it is high in late stage KS, HHV-8 is undetectable in some early lesions [23, 175, 176]that express detectable IC and DR expression [23]. Finally, as discussed below, IC can activate HHV-8 infection and increase viral load [88]. From these data it is tempting to speculate that the predominant role of HHV-8 is after initiation of KS.

As other herpesviruses, HHV-8 possesses several homologs of cellular genes including cytokines (v-IL-6), chemokine receptors (v-IL-8R), chemokines (v-MIP I, II and III) and potentially transforming genes like v-bcl-2 and v-cyclin D [177–179] (Table 5). However, most of these genes are expressed during lytic infection and not in latently infected KS spindle cells [180–185]. Only v-cyclin D expression can be detected by *in situ* hybridization in numerous KS spindle cells of late nodular KS lesions [186]. Nevertheless, recent data suggest that HHV-8 does not transform B cells or endothelial cells [187]. So

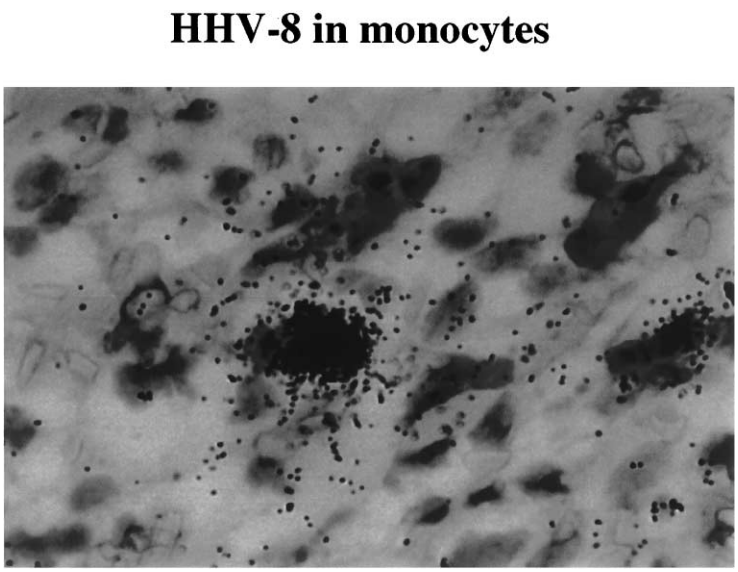

Figure 4. Detection of HHV-8 infected monocytes in KS lesions. Co-staining experiments with an antibody specific for monocytes-macrophages (anti-myeloid/histiocyte antigen, red staining) and *in situ* hybridization for detection of HHV-8 VP23 transcript (black grains) that is expressed only during HHV-8 viral lytic infection.

Table 5. Expression and activity of HIV and HHV-8 viral proteins in KS

Factor	Expression in KSC *in vitro*	Activity on KSC *in vitro*	Expression in KS lesions *in vivo*	Expression of cognate receptors in KSC *in vivo*	Possible role in KS pathogenesis
HIV-1 Tat	−	+ (P, CH,I)	+	+	Activates proliferation, migration, invasion, adhesion of E-KSC and IC-endothelial cells. Induces *in vitro* morphogenesis. Synergizes with IC or bFGF to enhance angiogenesis, KS cell invasion and growth, and aggressiveness of AIDS-KS. Increases bcl-2 expression. Binds KDR-1/activation. Increases HIV-1 replication and HHV-8 viral load. Activates IC production in HIV-1 infected cells and E-KSC or endothelial cells.
HHV-8-IL-6	−	n.d.	±	+ (gp130)	Can activate proliferation in the absence of IL-6R by direct interaction with gp130.
HHV-8-MIP-I	−	n.d.	±	n.d.	May contribute to the recruitment of monocytes and dendritic cells into the lesions.
HHV-8-CYCD	−	n.d.	+	n.d.	May be an accessory activator of KS spindle cell growth.

KSC, KS spindle cells; n.d., not done; (+) and (−) expression or activity present or absent, respectively; (±) weak expression; (P), proliferation; (CH), chemotaxis; (I), invasion.

HHV-8 may be an accessory activator of KS spindle cell growth possibly mediated by v-cyclin D [186] or may act indirectly by stimulating the expression of cellular factors with paracrine activity. On the other hand, the higher viral load found in KS patients and in late-nodular lesions suggest that individuals at risk of KS offer better conditions to virus growth and spread in the body. Recent evidence supports this hypothesis.

The same IC found increased in KS lesions can maintain and rescue viral growth, activate viral lytic replication and increase viral load in B cells and monocytes-macrophages [88], likely promoting viral transmission to other cell types. In addition, increased IC such as γIFN and DR activation can be found in early lesions prior to HHV-8 detection [23], suggesting that IC are, at least partially, responsible of virus growth and behavior in KS patients and in individuals at high risk to develop KS.

The data available indicate that circulating monocytes and derived cell types (macrophages, endothelial macrophages and dendritic cells) may play a key role in HHV-8 infection, virus recruitment into tissues, lytic infection and transmission to other cell types, including endothelial cells. Finally, these data suggest that the CD8 cell infiltration and activation present in KS lesions and a further

amplification of IC production may be in response to or be enhanced by HHV-8, as found for other herpesviruses such as EBV [188]. Although more studies are needed to understand the role of HHV-8 in KS development, these results suggest that the virus-host interplay is mediated by the same IC inducing cell recruitment, endothelial cell activation, angiogenesis and KS cell growth.

HIV-1 Tat PROTEIN: A PROGRESSION FACTOR IN AIDS-KS

All factors described above including HHV-8 are present in all forms of KS. However, AIDS-KS is more frequent and has a more aggressive course than the other KS forms, including AKS that acquires the most aggressive course after HIV-1 infection. Again, AIDS patients have at least 300-fold higher probability to get KS than individuals with primary immunodeficiency. This suggests that HIV-1 itself may play a role in KS development. Recent studies indicate that the Tat protein of HIV may be responsible for the aggressive nature of AIDS-KS.

Tat is a transcriptional activator of viral gene expression produced early after infection and essential for virus replication [109, 189, 190]. During acute infection of T cells by HIV-1, Tat is released from the cells in an active form [190–192] and via a leaderless secretory pathway that is specific and resembles that of IL-1, bFGF and aFGF [192]. In addition to its effect on paracrine and autocrine virus replication, *tat* possesses other activities on cell functions and can affect the growth and survival of T cells, endothelial cells and E-KSC [109]. In addition, transgenic mice carrying the Tat gene form KS-like lesions that are more frequent in male mice as KS in humans [193] and according to the level of expression of the transgene, Tat can cause tumors of various cell origin [194].

After release, extracellular Tat is capable of inducing the growth, migration and invasion of E-KSC [43, 45, 50, 190, 191] and of IC-activated endothelial cells (Table 5) [36, 43–45]. Tat also induces endothelial cells to express collagenase IV of the 72KD-type that is known to be associated with angiogenesis and tumor growth [45, 50]. Finally, Tat induces E-KSC and endothelial cell adhesion and stimulates endothelial cells to undergo *in vitro* morphogenesis [44, 45]. These effects of Tat suggested earlier that it mimics the effect of ECM proteins such as fibronectin and vitronectin that are known to play a key role in endothelial cell survival, adhesion growth, invasion and angiogenesis [195].

However, all the effects of Tat on normal endothelial cells require a previous exposure of the cells to the same IC increased in KS patients (Tables 2 and 3) and, again, γIFN appears to play a major role in inducing responsiveness to Tat [23, 36, 43, 44]. For example, endothelial cells do not enter the cell cycle after Tat stimulation unless they are pre-exposed to IC [36]. This is due to both IC induction of the expression of $\alpha5\beta1$ and $\alpha v\beta3$ integrins that function as the receptors for Tat and to the induction of bFGF expression that, in turn, induces the same integrins [44, 102, 195, 196] and it is required for Tat-angiogenic effect [50]. In contrast, both $\alpha5\beta1$ and $\alpha v\beta3$ integrins and bFGF are constitutively expressed by E-KSC that respond to Tat in the absence of other stimuli [44, 102, 196].

Consistent with these data, inoculation of Tat alone in nude mice does not lead to angiogenesis, however, when Tat is inoculated in the presence of suboptimal (non lesion forming) amounts of bFGF or with heparin, it greatly enhances bFGF-mediated angiogenesis and KS-like lesion formation in terms of both number of mice developing lesions and intensity of the histological alterations including angiogenesis and spindle cell growth (Table 5) [50, 197]. Similar synergistic effects are observed by inoculating mice with combined IC and Tat since IC induce both integrins and bFGF expression [23, 102, 196].

Interestingly, Tat exerts this synergistic effect with bFGF but not with VEGF and recent studies suggest that this is due to the binding of Tat to bFGF-induced integrins ($\alpha5\beta1$ and $\alpha v\beta3$) and not to $\alpha v\beta5$ that is induced by VEGF [102, 196] and is involved in its angiogenic pathway [195]. Tat possesses two domains that mediate these effects: the basic region that mediates heparin binding and the RGD region that mediates binding to integrins. Tat is a strong heparin-binding factor and its basic sequence competes with bFGF for binding to heparan sulfate proteoglycans of the cell surface and ECM [192]. By this competitive effect Tat releases ECM-bound bFGF and maintains it in a soluble form [102, 196]. At the same time, the RGD region of Tat binds the $\alpha5\beta1$ and $\alpha v\beta3$ integrins [44], induces the phosphorylation of the focal adhesion kinase p125 FAK (BE unpublished data) and promotes cell adhesion (when Tat is coated onto plates) or growth, migration, and invasion (when Tat is added to the cells in a soluble form). However, bFGF released by Tat-basic region represents the final mediator of Tat-induced cell growth, whereas Tat-induced cell adhesion increases the growth response to bFGF [50]. In contrast, cell migration and invasion are mediated only by the RGD region of Tat, as shown by mapping studies with overlapping Tat peptides and specific anti-integrin antibodies [102, 196].

As mentioned above, E-KSC but not IC-activated endothelial cells, proliferate in response to RGD peptides alone. This is not a feature of normal cells and suggests integrin clustering or some other alterations of the integrin-mediated pathway(s) [102, 196].

Thus, Tat exerts its effects via a molecular mimicry of ECM proteins and by releasing bound bFGF through a heparin-binding effect. This is consistent with the role of ECM proteins in angiogenesis and tumor growth [195].

Extracellular Tat is detectable in AIDS-KS lesions (Fig. 5) [50]. In addition, endothelial and spindle cells of KS lesions express both bFGF and $\alpha5\beta1$ and $\alpha v\beta3$-Tat receptors and extracellular Tat co-stains with these receptors on spindle cells and activated vessels [50], suggesting that the mechanisms described here are operative *in vivo*

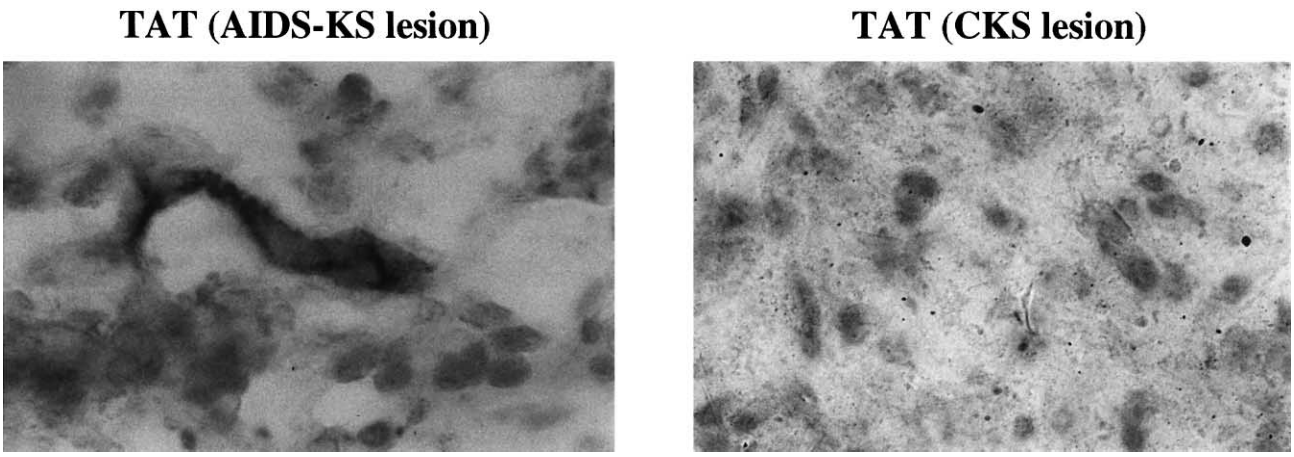

Figure 5. Expression of the HIV-1 Tat protein in AIDS-KS lesion. Tat is detected in all AIDS-KS lesions examined by immunohistochemistry with specific antibodies [50] and particularly in late lesions but not in CKS lesions (negative control). Double staining experiments indicate staining of Tat on activated endothelial cells and KS spindle cells and co-staining with $\beta 1$ and $\beta 3$ integrins [50].

and that Tat may explain the higher frequency and aggressiveness of KS in the setting of HIV-1 infection.

Tat has also been shown to activate the adhesion of monocytes-macrophages to the vessels with vascular damage due to the production of collagenases and to increase their migration and invasion into tissues [198].

Other data indicated that Tat basic region can bind KDR-1 [199], one of the VEGF receptors which has been shown to be expressed in KS lesions *in vivo* [112]. However, KS tumor cell lines but not E-KSC proliferate with VEGF but they all proliferate with Tat, suggesting that such a mechanism is more likely to occur in transformed-progressed KS given the receptor availability for Tat since VEGF is highly expressed in the lesions.

The other mechanisms by which Tat can affect KS development is through the activation of cellular gene expression and especially cytokine genes involved in KS pathogenesis [109] (Table 5). For example, Tat activates TNFα and β [200], IL-6 [201] and other genes in infected cells. As an extracellular protein Tat can induce TGFβ production in monocytes-macrophages [202], ELAM-1 expression in endothelial cells [203] and VCAM-1, ICAM-1, MCP-1 and IL-6 in E-KSC [204]. However, the concentration of extracellular Tat required for activation of cellular gene expression by Tat is generally higher (nano-micromolar) than that required for the effects mediated by integrins (picomolar) and described above [109, 190, 192]. This suggests that activation of cytokine genes by Tat is more likely to occur in an autocrine fashion (i.e. infected cells) than in a paracrine fashion (by extracellular Tat). However, Tat may contribute also by these mechanisms in AIDS-KS pathogenesis.

Other data suggest that Tat can increase also HHV-8 viral load [205], perhaps this is due to IC activation by Tat. Finally, Tat activates bcl-2 expression [206]. The presence of detectable extracellular Tat in sera from AIDS patients [207] and in AIDS-KS lesions [50] support the hypothesis of its role as a progression factor in AIDS-KS.

ONCOGENE EXPRESSION IN KS: bcl-2, A PROGNOSTIC MARKER OF PROGRESSION

Recent data indicate that bcl-2 is expressed in endothelial and spindle cells of the lesions from all forms of KS and that its expression increases with lesion stage reaching the maximal levels in nodular lesions [105, 208]. Bcl-2 is also induced during angiogenesis [195] suggesting that its expression may also be related to the angiogenic growth present in KS. The reasons for the induction of bcl-2 are under study, however, preliminary results suggest that the same IC and angiogenic factors present in KS lesions upregulate bcl-2 expression in endothelial and spindle cells (Sgadari *et al.*, in preparation). For example, as mentioned above, γIFN can contribute to induce bcl-2 expression by inducing the expression of CD40 [41]. Moreover, HIV-1 Tat protein can also induce bcl-2 expression [206].

The role of bcl-2 in KS is proven by the results of clinical trials of KS patients with taxol that have shown regression of KS [209]. Taxol, in fact, is known to inhibit bcl-2 function [210] and our unpublished work indicates that taxol blocks E-KSC growth and KS-like lesion formation in nude mice (Sgadari, in preparation). Thus, bcl-2 expression coupled with cell growth stimuli may divert cells from apoptosis toward continued cell proliferation and this may represent a step toward the lesion transformation and monoclonality that has been observed in some nodular KS lesions.

Besides bcl-2 only few other oncogens have been found to be expressed in KS. Among these the genes coding for *ras* [211] int-2 [212], p53 [208, 213, 214] and c-*myc* [215].

A significant over-expression of Ras protein has been observed in CKS [211] but without correlation with disease stage [211]. Int-2 (FGF-3) mRNA and protein have also been detected in KS [212]. However, the significance of these findings is yet unknown and requires further studies.

Heterozygous p53 mutations have been detected in KS

tissues [213] and p53 has also been detected by immunohistochemistry in late stage KS lesions but not in early lesions, however, very few cells (about 1%) of the lesions express detectable p53 [208, 214], suggesting that its role in KS pathogenesis may be limited.

C-*myc* expression is up-regulated by PDGF-B in cultured E-KSC and down-regulated by IFN-Con1, a derivative of IFN-α, which is cytostatic on E-KSC and has KS therapeutic effects [215]. Moreover, c-*myc*-specific antisense oligodeoxynucleotides inhibit specifically the proliferation and the migration of E-KSC *in vitro*. In addition, *in vivo* c-*myc* expression in spindle cells increases in late-nodular KS lesions as compared to early lesions [215]. This indicates that c-*myc* regulates KS spindle cell proliferation and migration and may have a key role in disease progression [215].

The expression of bcl-2, p53 and c-*myc* in late-nodular KS suggest that these proteins may be involved in KS progression and support the hypothesis that, in later stages of development, KS may transform from a reactive process to a true sarcoma.

CONCLUSIONS

The data reviewed in this article suggest that KS starts as an inflammatory-angiogenic lesion initiated by IC. IC, in turn, induce production of angiogenic molecules, growth and chemotactic factors that mediate lesion formation. IC are increased in all patients at high risk to develop KS due to immunoactivation. Disease worsening or onset is also observed after administration of γIFN, IL-2 or TNFα to the patients.

IC activate vessels, induce endothelial cells to acquire the KS cell phenotype, induce leukocytes recruitment and differentiation of monocytes in macrophages, endothelial macrophages and dendritic cells. IC also promote KS spindle cell proliferation and angiogenesis by inducing angiogenic factor production. In addition, IC increase replication of HHV-8 and HIV-1.

HHV-8 seems to be required for further progression of all the different epidemiological forms of KS, whereas the Tat protein of HIV-1 is a progression factor for AIDS-KS and may be responsible for the higher aggressiveness of this form of the disease. Continuous stimulation of reactive spindle cells by IC, growth factors, HHV-8 and the Tat protein may sporadically be oncogenic and result in the transformation of reactive KS lesions to a real sarcoma. This is supported by the increased expression of oncogenic factors, especially bcl-2, in late stage KS lesions and by the observations of clonality in some nodular KS lesions (Fig. 6).

Histologically KS spindle cells are considered as the tumor cells of KS. However, in early KS lesions this cellular compartment is actually composed of different reactive cell types: (i) activated endothelial cells (E-KSC, the predominant cell type) and (ii) macrophages (M-KSC). It is not clear whether these cells may form in loco or derive from the circulating spindle cell progenitors which are HHV-8 infected, are commonly found in KS patients and can be induced in non KS patients by IC. Both models could explain how KS initiates simultaneously in several different sites of the body. However, recent evidence favors a recruitment in the lesion of blood-derived monocytes that differentiate in loco.

Cell transformation may occur at later stages of KS. As suggested by the development from KS lesions of 2 transformed cell lines that give tumors only in SCID mice. This also indicates that a profound immunodeficiency may be required for progression of KS to a real sarcoma and may be more common in AIDS-KS patients particularly from Africa, as suggested by the microsatellite instability detected in AIDS-KS but not in CKS.

Regarding the role of HHV-8, it is likely that this virus is transported secondarily into initiating foci of reactive KS lesions. IC activate HHV-8 infection and increase viral load. Therefore, HHV-8, in turn, may find in KS patients and in KS lesions an optimal milieu to grow and spread. In fact, although HHV-8 is present in patients without KS, a higher viral load is detected in KS patients and in late-nodular lesions and IC production in early KS can precede HHV-8 detection. Productively HHV-8 infected circulating spindle cell progenitors (of monocytic origin), monocytes, and lymphocytes may carry HHV-8 into KS lesions, differentiate under the effect of IC and transmit HHV-8 to other cell types such as endothelial cells, which are predominantly latently infected. In this scenario, a key role of HHV-8 may be the enhancement of the CD8 T-cell infiltration and activation which is commonly observed in KS lesions. For unknown reasons infiltrating CD8 T cells are unable of eliminating virus-infected cells, in contrast, they further secrete IC which activate proliferation of infected KS spindle cells and may also contribute to maintain a latent HHV-8 infection in these cells. In this context the mutual interaction between CD8 T cells and spindle cells that is mediated by IC may be key to both HHV-8 infection and KS development.

The complex interaction among these factors is not yet completely understood and requires further studies, particularly for the role of HHV-8 in lesion formation. However, it is important to recognize two stages of KS particularly for therapeutical intervention. Early-stage KS occurs in the absence of immunodeficiency, is mediated and supported by cytokines and can regress. In contrast, late-stage KS may be growth independent, associated with immunodeficiency, does not regress and it is often resistant to conventional therapies. A pathogenetic therapy targeting specific factors and the monitoring of markers associated with KS development or progression such as activation markers, levels of circulating spindle cells, bcl-2 expression, HHV-8 viral load and the grade of the immunodeficiency may have prognostic values and address disease treatment.

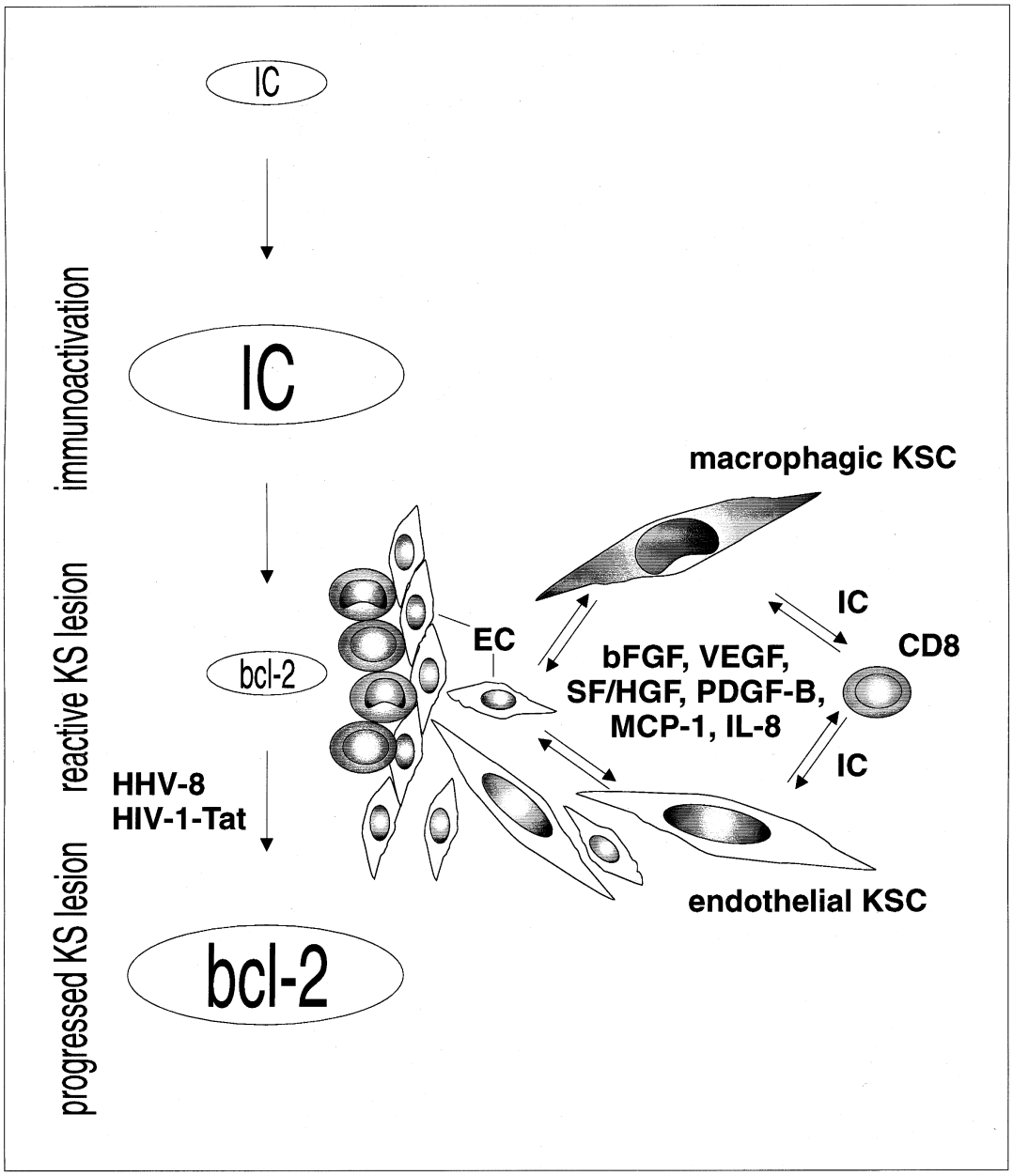

Figure 6. Schematic representation of key events mediating KS development. Immunoactivation increases the systemic levels of inflammatory cytokines (IC) including γIFN, IL-1, TNF. This causes endothelial cell activation (EC, black outline), increased expression of adhesion molecules by these cells (red outline) and increased adhesion of inflammatory cells including $CD8^+$ T cells (round nucleus) and monocytic/macrophagic cells (bean shaped nucleus) to the vessels. This supports their extravasation into the tissues and secretion of IC that these activated cells produce. Local IC cause phenotypic transformation of endothelial cells and monocytic/macrophagic cells to KS spindle cells (KSC). Alternatively or in addition to this event, IC induce the expansion of circulating spindle cell precursors (of monocytic origin) that may also be recruited by this mechanism into the tissues and differentiate to macrophagic/endothelial KSC (endothelial macrophages). Factors secreted by the spindle cells and inflammatory cells amplify these events and stimulate KSC proliferation (bFGF, PDGF), angiogenesis (bFGF, VEGF, SF/HGF, PDGF-B) and further recruitment of T cells, monocytes and other immune cells (MCP-1, IL-8). At the same time, HHV-8 infected monocytes-macrophages and spindle cell progenitors recruit the virus into tissues. Local IC upregulate HHV-8 infection and viral load creating a vicious cycle of virus-host interactions that amplify these events. In HIV-1 infected individuals, the Tat protein released in the circulation or in loco by HIV-infected cells binds KSC and activated EC and enhances KS cell growth, angiogenesis and bcl-2 expression and further upregulates IC production. In the course of this mutual stimulation among the different cell types, the oncogenic potential in KS lesions increases over time as indicated by the increased expression of proto-oncogenes (e.g. bcl-2) in advancing KS lesions. In the presence of immunodeficiency and, particularly, in HIV-1 infected individuals this may cause the transformation of a reactive and potentially reversible early KS lesion to a true sarcoma.

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REFERENCES

1. Friedman-Kien AE. Disseminated Kaposi's sarcoma syndrome in young homosexual men. *J Am Acad Dermatol* 1981, **5,** 468–471.
2. Haverkos HW, Drotman DP. Prevalence of Kaposi's sarcoma among patients with AIDS. *New Engl J Med* 1985, **312,** 1518.
3. Safai B, Johnson KG, Myskowski PL, *et al*. The natural history of Kaposi's sarcoma in the acquired immunodeficiency syndrome. *Ann Int Med* 1985, **103,** 744–750.
4. Slavin G, Cameron HM, Singh H. Kaposi's sarcoma in mainland Tanzania: a report of 117 cases. *Brit J Cancer* 1969, **23,** 349–357.
5. Taylor JF, Smith PG, Bull D, Pike MC. Kaposi's sarcoma in Uganda: geographic and ethnic distribution. *Brit J Cancer* 1972, **6,** 483–497.
6. Geddes M, Franceschi M, Balzi D, Arniani S, Gafà L, Zanetti R. Birthplace and classic Kaposi's sarcoma in Italy. *J Natl Cancer Inst* 1995, **87,** 1015–1017.
7. Franceschi S, Geddes M. Epidemiology of classic Kaposi's sarcoma, with special reference to Mediterranean population. *Tumori* 1995, **81,** 308–314.
8. Penn I. Kaposi's sarcoma in organ transplant recipients: report of 20 cases. *Transplantation* 1979, **27,** 8–11.
9. Trattner A, Hodak E, David M, Sandbank M. The appearance of Kaposi's sarcoma during corticosteroid therapy. *Cancer* 1993, **72,** 1779–1783.
10. Brooks JJ. Kaposi's sarcoma: a reversible hyperplasia. *Lancet* 1986, **2,** 1309–1311.
11. Levy JA, Ziegler JL. Acquired immunodeficiency syndrome is an opportunistic infection and Kaposi's sarcoma results from secondary immune stimulation. *Lancet* 1983, **2,** 78–81.
12. Real F, Krown SE. Spontaneous regression of Kaposi's sarcoma in patients with AIDS. *N Engl J Med* 1985, **313,** 1659.
13. Ensoli B, Salahuddin SZ, Gallo RC. AIDS-associated Kaposi's sarcoma: A molecular model for its pathogenesis. *Cancer Cells* 1989, **1,** 93–96.
14. Ensoli B, Barillari G, Gallo RC. Pathogenesis of AIDS-related Kaposi's sarcoma. *Hematol Oncol Clin North Am* 1991, **5,** 281–295.
15. Ensoli B, Gallo RC. Growth factors in AIDS-associated Kaposi's sarcoma: cytokines and HIV-I Tat protein. In DeVita V Jr, Hellman S, Rosenberg SA, eds. *AIDS Updates*. Philadelphia, J.B. Lippincott Co., 1994, **7,** 1–12.
16. Roth WK, Brandstetter H, Stürzl M. Cellular and molecular features of HIV-associated Kaposi's sarcoma. *AIDS* 1992, **6,** 895–913.
17. Ruszczak Z, Mayer-Da Silva A, Orfanos CE. Angioproliferative changes in clinically noninvolved, perilesional skin in AIDS-associated Kaposi's sarcoma. *Dermatologica*, 1987, **175,** 270–279.
18. Ruszczak Z, Mayer-Da Silva A, Orfanos CE. Kaposi's sarcoma in AIDS. *Am J Dermatopath* 1987, **9,** 388–398.
19. McNutt NS, Fletcher V, Conant MA. Early lesions of Kaposi's sarcoma in homosexual men. An ultrastructural comparison with other vascular proliferations in skin. *Am J Pathol* 1983, **111,** 62–77.
20. Dorfman RF, Path FRC. The histogenesis of Kaposi's sarcoma. *Lymphology* 1984, **17,** 76–77.
21. Ensoli B, Gallo RC. AIDS-associated Kaposi's sarcoma: A new perspective of its pathogenesis and treatment. *Proc Assoc Am Physicians* 1995, **107,** 8–18.
22. Stürzl M, Brandstetter H, Roth WK. Kaposi's sarcoma: a review of gene expression and ultrastructure of KS spindle cells *in vivo*. *AIDS Res Hum Retrov* 1992, **8,** 1753–1763.
23. Fiorelli V, Gendelman R, Sirianni MC, *et al*. γ-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.
24. MacPhail LA, Dekker NP, Regezi JA. Macrophages and vascular adhesion molecules in oral Kaposi's sarcoma. *J Cutaneous Pathol* 1996, **23,** 464–472.
25. Regezi SA, MacPhail LA, Daniels TE, De Souza 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, **43,** 240–249.
26. Nickoloff BJ, Griffiths CEM. Factor XIIIa-expressing dermal dendrocytes in AIDS-associated cutaneous Kaposi's sarcomas. *Science* 1989, **243,** 1736–1737.
27. 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.
28. 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.
29. Tabata M, Langford A, Becker J, Reichart PA. Distribution of immunocompetent cells in oral Kaposi's sarcoma (AIDS). *Oral Oncol* 1993, **29B,** 209–213.
30. Sirianni MC, Vincenzi L, Fiorelli V, *et al*. γ-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.
31. 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.
32. Little D, Said W, Siegel RJ, Fealy M, Fishbein MC. Endothelial cell markers in vascular neoplasms: an immunohistochemical study comparing factor VIII-related antigen, blood group specific antigens, 6-keto-PGF1α, and Ulex Europaeus 1 lectin. *J Pathol* 1986, **149,** 89–95.
33. Kraffert C, Planus L, Penneys NS. Kaposi's sarcoma: further immunohistologic evidence of a vascular endothelial origin. *Arch Dermatol* 1991, **127,** 1734–1735.
34. Gendelman R, Fiorelli V, Kao V, Gallo RC, Ensoli B. Spindle cells from both AIDS-associated and classical Kaposi's sarcoma (KS) lesions are of endothelial cell origin and present an activated phenotype. *AIDS Res Hum Retrov* 1994, **10,** S101.
35. Kaaya EE, Parravicini C, Ordonez C, *et al*. Heterogeneity of spindle cells in Kaposi's sarcoma: comparison of cells in lesions and culture. *J Acq Immun Def Synd Hum Retrov* 1995, **10,** 295–305.
36. Fiorelli V, Markham P, Gendelman R, 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.
37. 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.

38. Huang YQ, Friedman-Kien AE, Li JJ, Nickoloff BJ. Cultured Kaposi's sarcoma cell lines express factor XIIIa, CD14, and VCAM-1, but not factor VIII or ELAM-1. *Arch of Dermatol* 1993, **129,** 1291–1296.
39. Corbeil J, Evans LA, Vasak E, Cooper DA, Penny R. Culture and properties of cells derived from Kaposi sarcoma. *J Immunol* 1991, **146,** 2972–2976.
40. Zhang YM, Bachmann S, Hemmer C, *et al*. Vascular origin of Kaposi's sarcoma: expression of leucocyte endothelial adhesion molecule-1, thrombomodulin, and tissue factor. *Am J Pathol* 1994, **144,** 51–59.
41. 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.
42. Nakamura S, Salahuddin SZ, Biberfeld P, *et al*. Kaposi's sarcoma cells: long-term culture with growth factor from retrovirus-infected $CD4^+$ T cells. *Science* 1988, **242,** 427–430.
43. 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.
44. Barillari G, Gendelman R, Gallo RC, Ensoli B. The Tat protein of human immunodeficiency virus type 1, a growth factor for AIDS Kaposi 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.
45. Albini A, Barillari G, Benelli R, Gallo RC, Ensoli B. Angiogenic properties of human immunodeficiency virus type 1 Tat protein. *Proc Natl Acad Sci USA* 1995, **92,** 4838–4842.
46. 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.
47. 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.
48. Salahuddin SZ, Nakamura S, Biberfeld P, *et al*. Angiogenic properties of Kaposi's sarcoma-derived cells after long-term culture *in vitro*. *Science* 1988, **242,** 430–433.
49. 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.
50. Ensoli B, Gendelman R, Markham P, *et al*. Synergy between basic fibroblast growth factor and human immunodeficiency virus type 1 Tat protein in induction of Kaposi's sarcoma. *Nature* 1994, **371,** 674–680.
51. 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 oligonucleotides targeting basic fibroblast growth factor: a novel strategy for the therapy of KS. *J Clin Invest* 1994, **94,** 1736–1746.
52. Lunardi-Iskandar Y, Gill P, Lam V, *et al*. A neoplastic cell line (KS Y-l) from Kaposi's sarcoma (KS): evidence that late stage KS can be a true malignancy. *J Natl Cancer Inst* 1995, **87,** 974–981.
53. 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.
54. Rabkin CS, Janz S, Lash A, *et al*. Monoclonal origin of multicentric Kaposi's sarcoma lesions. *New Engl J Med* 1997, **336,** 988–993.
55. Gill P, Tsai Y, Rao AP, Jones P. Clonality in Kaposi's sarcoma. *New Engl J Med* 1997, **337,** 570–571.
56. Bedi GC, Westra WH, Farzadegan H, Pitha PM, Sidransky D. Microsatellite instability in primary neoplasms from HIV^+ patients. *Nature Med* 1995, **1,** 65–68.
57. Maurice PD, Smith NP, Pinching AJ. Kaposi's sarcoma with benign course in a homosexual. *Lancet* 1982, **1,** 571.
58. Schlessinger MFN, 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 Immunop* 1994, **73,** 252–260.
59. Ballard HS. Disseminated Kaposi's sarcoma without lymphocyte abnormalities. *Arch Intern Med* 1985, **145,** 547.
60. DePaoli P, Caffau C, D'Andrea M, Tavio M, Tirelli U, Santini G. Serum levels of intercellular adhesion molecule 1 in patients with HIV-1 related Kaposi's sarcoma. *J Acq Immun Def Synd* 1994, **7,** 695–699
61. Jaffe HW, Choi K, Thomas PA, *et al*. National case-control study of Kaposi's sarcoma and *Pneumocyctis carinii* pneumonia in homosexual men: part 1, epidemiological results. *Ann Intern Med* 1983, **99,** 145–151.
62. Fan J, Bass HZ, Fahev JL. Elevated IFNγ and decreased IL-2 gene expression are associated with HIV-1 infection. *J Immunol* 1993, **151,** 5031–5040.
63. Hober D, Haque A, Wattre P, Beucaire G, Mouton Y, Capron A. Production of tumor necrosis factor γ (TNFα) and interleukin 1 (IL-1) in patients with AIDS. Enhanced level of TNFα is related to higher cytotoxic activity. *Clin Exp Immunol* 1989, **78,** 329–333.
64. 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-1-induced disease. *J Immunol* 1990, **145,** 4059–4064.
65. Lahdevirta J, Maury CPJ, Teppo AM, Repo H. Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome. *Am J Med* 1988, **85,** 289–291.
66. Lepe-Zanuga JL, Mansell TWA, Hersh EM. Idiopathic production of Interleukin-1 in acquired immunodeficiency syndrome. *J Clin Microbiol* 1987, **5,** 1695–1700.
67. Vyakarnam A, Matear P, Meager A, *et al*. Alterated production of tumor necrosis factors α and β and interferon γ by HIV-1 infected individuals. *Clin Exp Immunol* 1991, **84,** 109–115.
68. Caruso A, Gonzales R, Stellini R, Scalzini A, Peroni L, Turano A. Interferon-γ marks activated T lymphocytes in AIDS patients. *AIDS Res Hum Retrov* 1990, **6,** 899–940.
69. Hahn T, Schattner A, Handzel ZT, Levin S, Bentwich Z. Possible role of natural cytotoxic activity in the pathogenesis of AIDS. *Clin Immunol Immunopathol* 1989, **50,** 53–61.
70. Kalinkovich A, Livshits G, Engelmann H, *et al*. Soluble tumor necrosis factor receptors (sTNF-R) and HIV infection: correlation to $CD8^+$ lymphocytes. *Clin Exp Immunol* 1993, **93,** 350–355.
71. Master SP, Taylor JF, Kyalwazi SK, Ziegler JL. Immunologic studies in Kaposi's sarcoma in Uganda. *Brit Med J* 1970, **1,** 600–602.
72. Kestens L, Melbye M, Biggar RJ, *et al*. Endemic African Kaposi's sarcoma is not associated with immunodeficiency. *Int J Canc* 1985, **36,** 49–54.
73 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.
74. Fagiolo U, Cossarizza A, Scala E, *et al*. Increased cytokine production in mononuclear cells of healthy elderly people. *Eur J Immunol* 1993, **23,** 2375–2378.
75. Levin MJ, Murray M, Rotbart HA, Zerbe GO, White CJ, Hayward AR. Immune response of elderly individual to

live attenuated varicella vaccine. *J Infect Dis* 1992, **166,** 253–259.
76. Beckstead JH. Oral presentation of Kaposi's sarcoma in a patient without severe immunodeficiency. *Arch Pathol Lab Med* 1992, **116,** 543–545.
77. Aboulafia D, Miles SA, Saks SR, Mitsuyasu RT. Intravenous recombinant tumor necrosis factor in the treatment of AIDS-related Kaposi's sarcoma. *J Acq Immun Def Synd* 1989, **2,** 54–58.
78. Albrecht H, Stellbrink H-J, Gross G, Berg B, Helmchen U, Mensing H. Treatment of atypical leishmaniasis with interferon γ resulting in progression of Kaposi's sarcoma in an AIDS patient. *Clin Invest* 1994, **72,** 1041–1047.
79. Krigel RL, Odajnyk CM, Laubenstein LJ, *et al*. Therapeutic trial of interferon γ in patients with epidemic Kaposi's sarcoma. *J Biol Response Mod* 1985, **4,** 358–364.
80. Krigel RL, Padavic-Shaller KA, Rudolph AR, Poiesz BJ, Comis RL. Exacerbation of epidemic Kaposi's sarcoma with a combination of interleukin-2 and γ interferon: results of a phase 2 study. *J Biol Response Mod* 1989, **8,** 359–365.
81. Mitsuyasu RT. Clinical aspects of AIDS-related Kaposi's sarcoma. In Abeloff MD, Klastersky J, eds. *Current Opinion in Oncology*, Baltimore, Current Science, 1993, **5,** 835–844.
82. Cotran RS, Pober JS. Endothelial activation: its role in inflammatory and immune reaction. In Simionescu N, Simionescu M, eds. *Endothelial Cell Biology*. New York, Plenum Publishing, 1988, 335–347.
83. Pober JS, Cotran RS. Cytokines and endothelial cell biology. *Phys Rev* 1990, **70,** 427–451.
84. Tannenbaum SH, Gralnick HR. γ-interferon modulates von Willenbrand factor release by cultured human endothelial cells. *Blood* 1990, **75,** 2177–2184.
85. Zietz C, Hotz B, Stürzl M, Rauch E, Penning R, Löhrs U. Aortic endothelium in HIV-1 infection: chronic injury, activation and increased leukocyte adherence. *Am J Pathol* 1996, **149,** 1887–1898.
86. Browning PJ, Sechler JMG, 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.
87. 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.
88. Colombini S, Monini P, Stürzl H, *et al*. The inflammatory cytokines increased in Kaposi's sarcoma reactivate human herpesvirus-8 infection in B cells and monocytes and promote the development of latently infected spindle cells. Submitted.
89. Lampugnani MG, Resnati M, Raitieri M, *et al*. A novel endothelial-specific membrane protein is a marker of cell-cell contacts. *J Cell Biol* 1992, **118,** 1511–1522.
90. Miles SA, Rezai AR, Salazar-Gonzalez JF, *et al*. AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6. *Proc Natl Acad Sci USA* 1990, **87,** 4068–4072.
91. Oxholm A, Oxholm P, Permin H, Bendtzen K. Epidermal tumour necrosis factor (α) and interleukin 6-like activities in AIDS-related Kaposi's sarcoma. *Acta Pathol Mic Sc* 1989, **97,** 533–538.
92. Stürzl M, Brandstetter H, Zietz C, *et al*. Identification of interleukin-1 and platelet-derived growth factor-β as major mitogens for the spindle cells of Kaposi's sarcoma: a combined *in vitro* and *in vivo* analysis. *Oncogene* 1995, **10,** 2007–2016.
93. Yang J, Hagan M, Offerman MK. Induction of IL-6 gene expression in Kaposi's sarcoma cells. *J Immunol* 1994, **152,** 943–955.
94. Ensoli B, Barillari G, Gallo RC. Cytokines and growth factors in the pathogenesis of AIDS-associated Kaposi's sarcoma. *Immunol Rev* 1992, **66,** 147–155.
95. Faris M, Ensoli B, Kokot N, Nel AE. Inflammatory cytokines induce the expression of bFGF isoforms required for growth of Kaposi's sarcoma and endothelial cells through the activation of AP-1 response elements of the bFGF promoter. *AIDS* in press.
96. Nair BC, DeVico AL, Nakamura S, *et al*. Identification of a major growth factor for AIDS-Kaposi's sarcoma cells as oncostatin M. *Science* 1992, **255,** 1430–1432.
97. Miles SA, Martinez-Maza O, Rezai A, *et al*. Oncostatin M as a potent mitogen for AIDS-KS-derived cells. *Science* 1992, **255,** 1432–1434.
98. Cai J, Gill PS, Masood P, *et al*. Oncostatin-M is an autocrine growth factor in Kaposi's sarcoma. *Am J Pathol* 1994, **145,** 74–79.
99. Faris M, Ensoli B, Stahl N, *et al*. Differential activation of the ERK, Jun Kinase and Janus Kinase-Stat pathways by oncostatin M and basic fibroblast growth factor in AIDS-derived Kaposi's sarcoma cells. *AIDS* 1996, **10,** 369–378.
100. Wijelath Es, Carisen B, Cole T, Chen J, Kotharl S, Hammond WP. Oncostatin M induces basic fibroblast growth factor expression in endothelial cells and promotes endothelial cell proliferation, migration and spindle morphology. *J Cell Sci* 1997, **100,** 871–879.
101. Takashima S, Klagsbrun M. Inhibition of endothelial cell growth by macrophage-like U-937 cell derived oncostatin M, leukemia inhibitory factor, and transforming growth factor β1. *J Biol Chem* 1996, **271,** 24901–24906.
102. Barillari G, Gendelman R, Fiorelli V, *et al*. HIV-1 Tat protein promotes angiogenesis triggered with inflammatory cytokines by retrieving heparin-bound basic fibroblast growth factor and by engaging the $\alpha v\beta 3$ integrin. Submitted.
103. Montesano R, Orci L, Vassalli P. Human endothelial cell cultures: phenotypic modulation by leukocyte interleukins. *J Cell Physiol* 1985, **122,** 424–434.
104. Liu YJ, Mason DY, Johnson GD, *et al*. Germinal center cells express bcl-2 protein after activation by signals which prevent their entry into apoptosis. *Eur J Immunol* 1991, **21,** 1905–1910.
105. Bohan-Morris C, Gendelman R, Marrogi AJ, *et al*. Immunohistochemical detection of bcl-2. in AIDS-associated and classical Kaposi's sarcoma. *Am J Pathol* 1996, **148,** 1055–1063.
106. Felipe Samaniego, Phillip D. Markham, Rita Gendelman, *et al*. Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor Are Expressed in Kaposi's Sarcoma and Synergize to induce Angiogenesis, Vascular Permeability and KS Lesion Development: Induction by Inflammatory Cytokines. *Am. J. Pathol*. (in press).
107. Weindel K, Marmé D, Weich HA. AIDS associated Kaposi's sarcoma cells in culture express vascular endothelial growth factor. *Biochem Bioph Res Co* 1992, **183,** 1167–1174.
108. 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.
109. Chang H-K, Gallo RC, Ensoli B. Regulation of cellular gene expression and function by the human immunodeficiency virus type 1 Tat protein. *J Biomed Sci* 1995, **2,** 189–202.
110. Xerri L, Hausson J, Planche J, *et al*. Fibroblast growth factor gene expression in AIDS—Kaposi's sarcoma detected by *in situ* hybridization. *Am J Pathol* 1991, **138,** 9–15.
111. Masood R, Cai J, Zheng T, Smith DL, Naidu Y, Gill PS.

Vascular endothelial growth factor/vascular permeability factor is an autocrine growth for AIDS—Kaposi's sarcoma. *Proc Natl Acad Sci USA* 1997, **94,** 979–984.
112. Brown LF, Tognazzi, K, Dvorak HF, Harrist TJ. Strong expression of kinase insert domain-containing receptor, a vascular permeability factor/vascular endothelial growth factor receptor in AIDS-associated Kaposi's sarcoma and cutaneous angiosarcoma. *Am J Pathol* 1996, **148,** 1065–1074.
113. Rosen EM, Goldberg ID. Scatter factor and angiogenesis. *Adv Cancer Res* 1995, **67,** 257–279.
114. Naidu YM, Rosen EM, Zitnick R, *et al*. Role of scatter factor in the pathogenesis of AIDS-related Kaposi's sarcoma. *Proc Natl Acad Sci USA* 1994, **91,** 5281–5285.
115. Maier JA, Mariotti M, Albini A, *et al*. Over-expression of hepatocyte growth factor in human Kaposi's sarcoma. *Int J Cancer* 1996, **65,** 168–172.
116. Roth WK, Werner S, Schirren CG, Hofschneider PH. Depletion of PDGF from serum inhibits growth of AIDS-related and sporadic Kaposi's sarcoma cells in culture. *Oncogene* 1989, **4,** 483–487.
117. Werner S, Hofschneider PH, Heldin CH, Ostman A, Roth WK. Cultured Kaposi's sarcoma-derived cells express functional PDGF-A type and B-type receptors. *Exp Cell Res* 1990, **187,** 98–103.
118. 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.
119. Brown DM, Hong SP, Farell CL, Pierce GF, Khouri RK. Platelet-derived growth factor BB induces functional vascular anastomoses *in vivo*. *Proc Natl Acad Sci USA* 1995, **92,** 5920–5924.
120. Mantovani A, Bussolino F, Introna M. Cytokine regulation of endothelial cell function: from molecular level to the bedside. *Immunol Today* 1997, **18,** 231–240.
121. Murakami-Mori K, Taga T, Kishimoto T, Nakamura S. AIDS-associated Kaposi's sarcoma (KS) cell express oncostatin M (OM)-specific receptor but not leukemia inhibitory factor/OM receptor or interleukin-6. receptor. *J Clin Invest* 1995, **96,** 1319–1327.
122. Murakami-Mori K, Taga T, Kishimoto T, Nakamura S. The soluble form of IL-6 receptor (sIL-6R α) is a potent growth factor for AIDS-associated Kaposi's sarcoma (KS) cells; the soluble form of gp130. is antagonistic for sIL-6R α-induced AIDS-KS cell growth. *International Immunol* 1996, **8,** 595–602.
123. 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.
124. Stürzl M, Cornali E, Gahlemann C, *et al*. Expression of growth factors, cytokines, and chemokines in Kaposi's sarcoma in HIV and cytokines. M. Guneounou, eds. Les Editions INSERM, 1997, 349–357.
125. Bussolino F, Wang JM, De Filippi P. Granulocyte and granulocyte-macrophages colony stimulating factors induce human endothelial cells to migrate and proliferate. *Nature* 1989, **337,** 471–473.
126. Clark SC, Kamen R. The human hematopoietic colony stimulating factors. *Science* 1987, **236,** 1229–1236.
127. Werner PH, Hofschneider PH, Stürzl M, Dick I, Roth WK. Cytochemical and molecular properties of simian virus 40 transformed Kaposi's sarcoma-derived cells: evidence for the secretion of a member of the fibroblast growth factor family. *J Cell Physiol* 1989, **141,** 490–502.
128. Li JJ, Huang YQ, Moscatelli D, Nicolaides A, Zhang WC, Friedman-Kien AE. Expression of fibroblast growth factors and their receptors in acquired immunodeficiency syndrome-associated Kaposi's sarcoma tissue and derived cells. *Cancer* 1993, **72,** 2253–2259.
129. Roth WK, Werner S, Hofschneider PH. Autocrine and paracrine growth stimulation of Kaposi's sarcoma derived cell cultures. *VI International Conference on AIDS*. San Francisco, 1990, Abstract ThA321.
130. Bussolino F, Arese M, Montrucchio G, *et al*. Platelet activating factor produced *in vitro* by Kaposi's sarcoma cells induces and sustains *in vivo* angiogenesis. *J Clin Invest* 1995, **96,** 940–952.
131. Werner S, Viehweger P, Hofschneider PH, Roth WK. Low mitogenic response to EGF and TGF-α: A characteristics feature of cultured Kaposi's sarcoma derived cells. *Oncogene* 1991, **6,** 59–64.
132. Roth WK. TGF-β and FGF-like growth factors involved in the pathogenesis of AIDS-associated Kaposi's sarcoma. *Res Virol* 1993, **144,** 105–109.
133. Williams AO, Ward JM, LI JF, Jackson MA, Flanders KC. Immunohistochemical localization of transforming growth factor-β 1 in Kaposi's sarcoma. *Hum Pathol* 1995, **26,** 469–473.
134. Chang Y, Cesarman E, Pessin MS, *et al*. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 1994, **266,** 1865–1869.
135. 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.
136. Boshoff C, Acholz T, Kennedy MM, *et al*. Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. *Nature Med* 1995, **1,** 1274–1278.
137. Schalling M, Ekman M, Kaaya EE, Linde A, Biberfeld P. A role for a new herpesvirus (KSHV) in different forms of Kaposi's sarcoma. *Nature Med* 1995, **1,** 707–708.
138. Huang YQ, Li JJ, Kaplan MH, *et al*. Human herpesvirus-like nucleic acid in various forms of Kaposi's sarcoma. *Lancet* 1995, **345,** 759–762.
139. Buonaguro FM, Tornesello ML, Beth-Giraldo E, *et al*. Herpesvirus-like DNA sequences detected in endemic, classic, iatrogenic and epidemic Kaposi's sarcoma (KS) biopsies. *Int J Cancer* 1996, **65,** 25–28.
140. Albini A, Aluigi MG, Benelli R, *et al*. Oncogenesis in HIV-infection: KSHV and Kaposi's sarcoma. *Int J Oncol* 1996, **9,** 5–8.
141. Soulier J, Grollet L, Oksenhendler E, *et al*. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. *Blood* 1995, **86,** 1276–1280.
142. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N Engl J Med* 1995, **332,** 1186–1191.
143. Dupin N, Gorin I, Deleuze J, Agut H, Huraux J-M, Escande J-P. Herpes-like DNA sequences, AIDS-related tumors, and Castleman's disease. *N Engl J Med* 1995, **333,** 799.
144. Otsuki T, Kumar S, Ensoli B, *et al*. Detection of HHV-8/KSHV DNA sequences in AIDS-associated extranodal lymphoid malignancies. *Leukemia* 1996, **10,** 1358–1362.
145. Rettig MB, Ma HJ, Vascio RA, *et al*. Kaposi's sarcoma-associated herpesvirus infection of bone marrow dendritic cells from multiple myeloma patients. *Science* 1997, **276,** 1851–1854.
146. Kedes DH, Operskalski E, Busch M, Kohn R, Fllod 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. *Nature Med* 1996, **2,** 918–924.
147. 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.

148. Rezza G, Lennette ET, Giuliani M, *et al*. Prevalence and determinants of anti-lytic and anti-latent antibodies to HHV-8 among Italian individuals at risk of sexually and parenterally transmitted infections. Submitted.
149. Monini P, De Lellis L, Fabris M, Rigolin F, Cassai E. Kaposi's sarcoma herpesvirus (KSHV) is a ubiquitous virus frequently present in prostate tissue and human sperm. *N Engl J Med* 1996, **334,** 1168–1172.
150. Blackbourn DJ, Ambroziak J, Lennette E, Adams M, Ramachandran B, Levy JA. Infectious human herpesvirus 8 in a healthy North American blood donor. *Lancet* 1997, **349,** 609–611.
151. De Lellis L, Fabris M, Cassai E, *et al*. Herpesvirus-like DNA sequences in non-AIDS Kaposi's sarcoma. *J Infect Dis* 1995, **172,** 1605–1607.
152. Bigoni B, Dolcetti R, De Lellis L, *et al*. Human Herpesvirus 8 is present in the lymphoid system of healthy persons and can reactivate in the course of AIDS. *J Infec Dis* 1996, **173,** 542–549.
153. Viviano E, Vitale F, Ajello F, *et al*. Human herpesvirus type 8 DNA sequences in biological samples of HIV-positive and negative individuals in Sicily. *AIDS* 1997, **11,** 607–612.
154. Corbellino M, Poirel L, Bestetti G, *et al*. Restricted tissue distribution of extralesional Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS patients with Kaposi's sarcoma. *AIDS Res Human Retrov* 1996, **12,** 651–657.
155. Decker LL, Shankar P, Khan G, *et al*. The Kaposi's 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.
156. Huang J-O, Feiner D, Zhang WG, Cockerell CJ, Demopoulos RD, Friedman-Kien AE. Detection of HHV-8 in tissues of various organs from AIDS-KS patients. *Abstract Book of the XI Int. Conference on AIDS*. Vancouver 1996, **2,** 8.
157. Gao S, 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.
158. 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.
159. Rizzieri DA, Liu J, Miralles D, Traweek ST. Kaposi's sarcoma-associated herpesvirus is detected in peripheral blood mononuclear cells of HIV-infected homosexuals more often than in heterosexuals. *Cancer J Sci Am* 1997, **3,** 153–156.
160. Purvis SF, Katongole-Mbidde E, Johnson JL, *et al*. High incidence of Kaposi's sarcoma-associated herpesvirus and Epstein–Barr virus in tumor lesions and peripheral blood mononuclear cells from patients with Kaposi's sarcoma in Uganda. *J Infect Dis* 1997, **175,** 947–950.
161. Kasolo FC, Mpabalwani E, Gompels UA. Infection with AIDS-related herpesviruses in human immunodeficiency virus-negative infants and endemic childhood Kaposi's sarcoma in Africa. *J Gen Virol* 1997, **78,** 847–855.
162. Moore PS, Kingsley L, Holmberg SD, *et al*. Kaposi's sarcoma-associated herpesvirus infection prior to onset of Kaposi's sarcoma. *AIDS* 1996, **10,** 175–180.
163. 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,** 799–803.
164. Mesri EA, Cesarman E, Arvanitakis L, *et al*. Human herpesvirus-8/Kaposi's sarcoma-associated herpesvirus is a new transmissible virus that infects B cells. *J Exp Med* 1995, **183,** 2385–2390.
165. Harrington MJ, 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.
166. Blasig C, Zietz C, Haar B, *et al*. Monocytes in Kaposi's sarcoma lesions are productively infected by human herpesvirus-8. *J Virol* 1997, **10,** 7963–7968.
167. 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 (KS). *Int J Cancer* 1997, **72,** 68–71.
168. Boshoff C, Whitby D, Hatzioannou T, *et al*. Kaposi's sarcoma-associated herpesvirus in HIV-negative Kaposi's sarcoma. *Lancet* 1995, **345,** 1043–1044.
169. Li JJ, Huang YQ, Cockerell CJ, Friedman-Kien AE. Localization of human herpes-like virus type 8 in vascular endothelial cells and perivascular spindle-shaped cells of Kaposi's sarcoma lesions by *in situ* hybridization. *Am J Pathol* 1996, **148,** 1741–1748.
170. 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.
171. Rainbow L, Platt GM, Simpson GR, *et al*. The 222- to 23-kilodalton latent nuclear protein (LNA) of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) is encoded by orf 73 and is a component of the latency-associated nuclear antigen. *J Virol* 1997, **71,** 5915–5921.
172. 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.
173. Dictor M, Rambech E, Way D, Witte M, Bendsoe N. Human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) DNA in Kaposi's sarcoma lesions, AIDS Kaposi's sarcoma cell lines, endothelial Kaposi's sarcoma simulators, and the skin of immunosuppressed patients. *Am J Pathol* 1996, **148,** 2009–2016.
174. Lebbé C, de Crémoux P, Rybojad M, *et al*. Kaposi's sarcoma and new herpesvirus. *Lancet* 1995, **345,** 1180.
175. Maiorana A, Luppi M, Barozzi P, Collina G, Fano RA, Torelli G. Detection of human herpes virus type 8 DNA sequences as a valuable aid in the differential diagnosis of Kaposi's sarcoma. *Modern Pathol* 1997, **10,** 182–187.
176. Noel JC. Kaposi's sarcoma and KSHV. *Lancet* 1995, **346,** 1359.
177. Neipel F, Albrecht JC, Ensser A, *et al*. Human herpesvirus 8 encodes a homolog of interleukin-6. *J Virol* 1997, **71,** 839–842.
178. Neipel F, Albrecht J-C, Fleckenstein B. Cell-homologs genes in the Kaposi's sarcoma-associated rhadinovirus human herpesvirus 8; determinants of its pathogenicity. *J Virol* 1997, **71,** 4187–4192.
179. Russo JJ, Bohenzky RA, Chien MC, *et al*. Nucleotide sequence of the Kaposi's sarcoma-associated herpesvirus (HHV-8). *Proc Natl Acad Sci USA* 1996, **93,** 14862–14867.
180. Sarid R, Sato T, Bohenzky RA, Russo JJ, Chang Y. Kaposi's sarcoma-associated herpesvirus encodes a functional bcl-2 homologue. *Nature Med* 1997, **3,** 293–298.
181. Nicholas J, Cameron KR, Honess RH. Herpesvirus saimiri encodes homologues of G protein-coupled receptors and cyclins. *Nature* 1992, **355,** 362–365.
182. Cesarman E, Nador RG, Bai F, *et al*. Kaposi's sarcoma-associated herpesvirus contains G protein-coupled receptor and cyclin D homologs which are expressed in Kaposi's sarcoma and malignant lymphoma. *J Virol* 1996, **70,** 8218–8223.
183. Chang Y, Moore PS, Talbot SJ, *et al*. Cyclin encoded by KS herpesvirus. *Nature* 1996, **382,** 410.

184. 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.
185. Stürzl M, Ascherl G, Blasig C, Opalenik SR, Ensoli B, Browning PJ. Expression of the human herpesvirus 8 (HHV-8) encoded MIP-I gene (v-MIP-I) in Kaposi's sarcoma (KS) lesions. *AIDS* (in press).
186. Davis MA, Stürzl M, Blasig C, *et al*. Latent expression of human herpesvirus 8-encoded cyclin gene in Kaposi's sarcoma. *J Natl Cancer I* 1997, **89,** 1868–1874.
187. Boshoff C, Talbot S, Whitby D, Reeves J, Weiss RA. AIDS associated tumors and HHV8. *J Acqu Immun Def Synd Hum Retrov* 1997, **14,** S9.
188. Callan MF, Steven N, Krausa P, *et al*. Large clonal expansions of $CD8^+$ T cells in acute infectious mononucleosis. *Nature Med* 1996, **2,** 906–911.
189. Arya SK, Guo C, Josephs SF, Wong-Staal F. Transactivator gene of human T-lymphotropic virus type III (HTLV-III). *Science* 1985, **229,** 69–73.
190. 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.
191. Ensoli B, Barillari G, Salahuddin SZ, Gallo RC, Wong-Staal 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.
192. 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.
193. Vogel J, Hinrichs SH, Reynolds RK, Luciw PA, Jay G. The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. *Nature* 1988, **335,** 601–661.
194. Corallini A, Altavilla G, Pozzi L, *et al*. Systemic expression of HIV-1 tat gene in transgenic mice induces endothelial proliferation and tumors of different histotypes. *Cancer Res* 1993, **53,** 5569–5575.
195. Strömblad S, Cheresh DA. Cell adhesion and angiogenesis. *Cell Biol* 1996, **6,** 462–468.
196. Barillari G, Fiorelli V, Gendelman R, *et al*. HIV-1 Tat protein enhances angiogenesis and Kaposi's sarcoma (KS) development triggered by inflammatory cytokines (IC) or bFGF by engaging the $\alpha v\beta 3$ integrin. *J Acq Immun Def Synd Hum Retrov* 1997, **14,** A33.
197. Albini A, Fontanini G, Masiello L, *et al*. Angiogenic potential *in vivo* by Kaposi'sarcoma cell-free supernatants and HIV-1 Tat product: inhibition of KS-like lesions by tissue inhibitor of metalloproteinase-2. *AIDS* 1994, **8,** 1237–1244.
198. Lafrenie RM, Wahl LM, Epstein JS, Hewlett IK, Yamada KM, Dhawan S. HIV-1 Tat protein promotes chemataxis and invasive behavior by monocytes. *J Immunol* 1996, **157,** 974–977.
199. Albini A, Soldi R, Giunciuglio D, *et al*. The angiogenesis induced by HIV-1 Tat protein is mediated by the Flk/1/KDR receptor on vascular endothelial cells. *Nature Med* 1996, **2,** 1371–1375.
200. Buonaguro L, Buonaguro FM, Giraldo G, Ensoli B. The human immunodeficiency virus type 1 Tat protein transactivates tumor necrosis factor β gene expression through a TAR-like structure. *J Virol* 1994, **68,** 2677–2682.
201. Scala G, Ruocco MR, Ambrosino C, *et al*. The expression of the interleukin 6 gene is induced by the human immunodeficiency virus 1 Tat protein. *J Exp Med* 1994, **179,** 961–971.
202. Zauli G, Davis BR, Re MC, Visani G, Furlini G, La Placa M. Tat protein stimulates production of transforming growth factor-β 1 by marrow macrophages: a potential mechanism for human immunodeficiency virus-1-induced hematopoietic suppression. *Blood* 1992, **80,** 3036–3043.
203. Hofman FM, Wright AD, Dohadwala MM, Wong-Staal F, Walker SM. Exogenous Tat protein activates human endothelial cells. *Blood* 1993, **82,** 2774–2780.
204. Domingo Kelly G, Ensoli B, Gunthel CJ, Offermann MK. Induction of inflammatory response genes by purified Tat in Kaposi's sarcoma cells. Submitted.
205. Harrington W, Sieczkowski L, Sosa C, *et al*. Activation of HHV-8 by HIV-1 Tat. *Lancet* 1997, **349,** 774.
206. Zauli G, Gibellini D, Caputo A, *et al*. The human immunodeficiency virus type 1 Tat protein upregulates bcl-2 gene expression in Jurkat T-cell lines and primary peripheral blood mononuclear cells. *Blood* 1995, **86,** 3823–3834.
207. Westendorp MO, Frank R, Ochsenbauer C, *et al*. Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. *Nature* 1995, **275,** 497–500.
208. Montmayeur F, Krajewski S, Bejui-Thivolet F, *et al*. *In vivo* patterns of apoptosis-regulating protein expression in Kaposi's sarcoma. *Appl Immunohistohemistry* 1997, **5,** 104–110.
209. Saville MW, Lietzau J, Pluda JM, *et al*. Treatment of HIV-associated Kaposi's sarcoma with paclitaxel. *Lancet* 1995, **346,** 26–28.
210. Haldar S, Jena N, Croce CM. Inactivation of bcl-2. by phosphorylation. *Proc Natl Acad Sci USA* 1995, **92,** 4507–4511.
211. Spandidos DA, Kaloterakis A, Yiagnisis M, Varatsos A, Field JK. Ras, c-*myc* and c-erbB-2 oncoprotein expression in non-AIDS mediterrranean Kaposi's sarcoma. *Anticancer Res* 1990, **10,** 1619–1626.
212. Huang YQ, Li JJ, Moscatelli D, *et al*. Expresion of the int-2 oncogene in Kaposi's sarcoma lesions. *J Clin Invest* 1993, **91,** 1191–1197.
213. Scincariello F, Dolan MJ, Nedelcu I, Tyring SK, Hillard JK. Occurrence of human papillomavirus and p53 gene mutations in Kaposi's sarcoma. *J Virol* 1994, **203,** 153–157.
214. Bergman R, Ramon M, Kilim S, Lichtig C, Friedman-Birnbaum R. An immunohistochemical study of p53 protein expression in classical Kaposi's sarcoma. *Am J Dermatol* 1996, **18,** 367–370.
215. Köster R, Blatt LM, Streubert M, *et al*. Consensus-interferon and platelet-derived growth factor adversely regulate proliferation and migration of Kaposi's sarcoma cells by control of c-myc expression. *Am J Pathol* 1996, **149,** 1871–1885.