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 angiogenic-inflammation lesion mediated by inflammatory cytokines and angiogenic factors, that is triggered or amplified by infection with human herpesvirus-8. In addition, the human immuno-deficiency 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.

Key words: Kaposi’s sarcoma · inflammatory cytokines · angiogenic factors · CD8 T cells · monocytes-macrophages · circulating spindle cell progenitors · human herpesvirus-8 · HIV-1 Tat · AIDS · bel-2
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. Immunohistoch- emical 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 (MHC II+) 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 “trans-differentiated” 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 immunooactivation 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 [60–62]. Elderly men can present an oligoclonal CD8 expansion with increased production of IL-1 and tumor necrosis factor \( \alpha \) (TNF-\( \alpha \)) 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 (\( \gamma \)IFN 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 \( \gamma \)IFN 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 \( \gamma \)IFN, IL-1, TNF-\( \alpha \) appears to be key to KS development. In fact, the administration of \( \gamma \)IFN, IL-2 or TNF-\( \alpha \) 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

<table>
<thead>
<tr>
<th>Marker</th>
<th>Specificity</th>
<th>Cultured E-KSC</th>
<th>In situ E-KSC</th>
<th>Cultured M-KSC</th>
<th>In situ M-KSC</th>
<th>Cultured circulating KSC progenitors</th>
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<tbody>
<tr>
<td>FVIII-RA</td>
<td>Vascular endothelium</td>
<td>±⁴</td>
<td>±⁴</td>
<td>−</td>
<td>−</td>
<td>−³</td>
</tr>
<tr>
<td>CD34</td>
<td>Vascular endothelium and hematopoietic cell progenitors</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−³</td>
</tr>
<tr>
<td>VE-Cadherin</td>
<td>Vascular endothelium, endothelial macrophages</td>
<td>+</td>
<td>+</td>
<td>n.d.</td>
<td>n.d.</td>
<td>+</td>
</tr>
<tr>
<td>CD31</td>
<td>Macrophages, endothelial cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD14</td>
<td>Monocytes-macrophages</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD68</td>
<td>Tissue macrophages</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD36</td>
<td>Macrophage, capillary endothelium</td>
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<td>+</td>
<td>n.d.</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>PAM-1</td>
<td>Macrophages</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>CD45</td>
<td>Leukocytes</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD4</td>
<td>T cells, monocytes-macrophages</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Macrophages, activated endothelial cells, others</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Macrophages, activated endothelial cells, others</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>VCAM-1</td>
<td>Activated endothelial cells, others</td>
<td>+</td>
<td>+</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>ELAM-1</td>
<td>Activated endothelial cells</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>n.d.</td>
</tr>
<tr>
<td>CD40</td>
<td>Vascular endothelium</td>
<td>+</td>
<td>+</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>zβf1 and zβf3</td>
<td>Activated endothelial cells, others</td>
<td>+</td>
<td>+</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

E-KSC and M-KSC: endothelial- or macrophagic-KS spindle cells, respectively. (+), positive expression; (−), negative expression; n.d., not done.

*Positive after culture in the absence of TCM.

²Positive in early stage KS, lost in late stages.

³In a previous report few weakly positive cells have been detected [86].

⁴No 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
Several of the IC present in TCM (IL-1α 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 KS 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 KS 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, z5β1, zvβ3 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
CD39 is also highly expressed by KS spindle cells of
and Tables 1 and 2. In addition, contribute to these effects in a synergistic fashion (Fig. 1).

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

| Table 3 Expression and activity of inflammatory cytokines in KS |
|-----------------|-----------------|-----------------|-----------------|-------------------|
| Factor          | Expression in KSC | Activity on KSC in vitro | Expression in KS | Expression of cognate receptors in KSC | 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 (xαβ1 and xαβ3 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
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 1 and 3).

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.

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**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 3: Expression and activity of growth and angiogenic factors in KS

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

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 acidic 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 serological 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-bel-2 and v-cyclin D [177–179] (Table 5). However, most of these genes are expressed during lytic infection and are potentially lytically 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
**HHV-8 in monocytes**

![Image of HHV-8 infected monocytes](image)

**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.

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

<table>
<thead>
<tr>
<th>Factor</th>
<th>Expression in KSC</th>
<th>Activity on KSC in vitro</th>
<th>Expression in KS lesions</th>
<th>Expression of cognate receptors in KSC in vivo</th>
<th>Possible role in KS pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 Tat</td>
<td>−</td>
<td>+ (P, CH, I)</td>
<td>+</td>
<td>+</td>
<td>Activates proliferation, migration, invasion, adhesion of E-KSC and IC-endothelial cells. Induces <em>in vitro</em> 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.</td>
</tr>
<tr>
<td>HHV-8-IL-6</td>
<td>−</td>
<td>n.d.</td>
<td>±</td>
<td>+ (gp130)</td>
<td>Can activate proliferation in the absence of IL-6R by direct interaction with gp130. May contribute to the recruitment of monocytes and dendritic cells into the lesions.</td>
</tr>
<tr>
<td>HHV-8-MIP-1</td>
<td>−</td>
<td>n.d.</td>
<td>±</td>
<td>n.d.</td>
<td>May be an accessory activator of KS spindle cell growth.</td>
</tr>
<tr>
<td>HHV-8-CYCD</td>
<td>−</td>
<td>n.d.</td>
<td>+</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>

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

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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 z5β1 and zvβ3 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 z5β1 and zvβ3 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 (z5β1 and zvβ3) and not to zvβ5 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 z5β1 and zvβ3 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 z5β1 and zvβ3-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.
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
lesions and by the observations of clonality in some nodules of oncogenic factors, especially bcl-1, in late-stage KS sarcoma. This is supported by the increased expression in the transformation of reactive KS lesions to a real sarcoma 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-7 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 bel-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, bel-2 expression, HHV-8 viral load and the grade of the immunodeficiency may have prognostic values and address disease treatment.

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 immunodeficiency. 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 bel-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.
Figure 6. Schematic representation of key events mediating KS development. Immunoactivation increases the systemic levels of inflammatory cytokines (IC) including IFN, 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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Kaposi’s Sarcoma


