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Review

Interactions between endothelial cells and HIV-1

Federico Bussolino [a,*], Stefania Mitola [a], Guido Serini [a], Giovanni Barillari [b], Barbara Ensoli [b]

[a] *Department of Genetics, Biology and Biochemistry, Institute for Cancer Research and Treatment, School of Medicine, University of Torino, s.p. 142, Km. 395, 10060 Candiolo, Torino, Italy*

[b] *Laboratory of Virology, Istituto Superiore di Sanità, 00100 Roma, Italy*

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Abstract

Endothelial cells (EC) participate in inflammatory and immune reactions by producing and responding to soluble mediators. Human immunodeficiency virus (HIV)-1 profoundly alters the features of EC. In some anatomical districts, they are infected by the virus and may represent a relevant reservoir. During lymphomononuclear cell diapedesis, EC activate virus replication in crossing cells. Direct or indirect damage of EC is particularly relevant in central nervous system, where blood-brain barrier perturbation is pivotal in neuronal degeneration. The observed alterations of EC adhesive properties contribute in altered leukocyte traffic from blood to lymphoid organs and tissues and play a role in the onset of immune surveillance alteration. These alterations of EC functions are relevant for the general vasculopathy, which marks the acquired immunodeficiency syndrome, and in particular are instrumental in the pathogenesis of Kaposi's sarcoma. Here we discuss the biological and molecular activation of EC in HIV-1 infection that represents the basis to understand the pathogenesis of HIV-1 associated vascular diseases.

Keywords: Tat; Kaposi's sarcoma; Herpes virus 8; HIV infection; Tumors

Contents

* Corresponding author. Tel.: +39-011-9933347; fax: +39-011-9933524.
E-mail address: fbussolino@ircc.unito.it (F. Bussolino).

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1. Introduction

Localized between blood and tissues, endothelium represents a dynamic barrier that regulates in–out and out–in signals resulting in the control of body homeostasis. It contributes in blood rheology by its anti-thrombotic and pro-fibrinolytic features, in delivering nutrients to and removing wastes from tissues, and in traffic of blood cells. Physical forces, alteration of soluble mediator networks or of the circulating concentration of metabolites, enzymatic defects and host invasion induce perturbations of endothelial cells (EC) and cause changes of their genetic program. Therefore, EC assume a pro-thrombotic and a pro-inflammatory phenotype and actively participate in the immune response by assuming properties of antigen presenting cells and allowing leukocytes to migrate in injured tissues or cancer cells to metastasize. In adult life, activated EC may increase their proliferative rate and generate new vessels during tissue repair, growth of solid tumors, chronic inflammatory and metabolic diseases [1,2].

Human immunodeficiency virus (HIV)-1, the etiologic agent of acquired immunodeficiency syndrome (AIDS), causes several vascular disorders characterized by an evident activation and perturbation of EC. These include vasculitis in several organs [3–7] but in particular in central and peripheral nervous system [8–10], serum protein leakage across the blood-brain barrier [11], thrombotic episodes [12–14], and enhanced transendothelial migration of HIV-infected monocytes in the brain that contributes to HIV-1-associated encephalitis [15,16]. In addition, recent evidence indicates the presence of severe morphologic alterations of the aortic endothelium in HIV-1-infected patients that are associated with EC activation and increased adhesion of mononuclear cells [17]. Finally, infected patients show a high susceptibility to manifest bacillary angiomatosis, an abnormal systemic vascular proliferation with nodule formation characterized by prominent angiogenesis and neutrophil infiltration, caused by the Gram-negative coccobacillus *Bartonella henselae* [18,19].

However, the most profound mark of AIDS-associated vasculopathy is Kaposi's sarcoma (KS). KS is the most frequent tumor of HIV-1-infected individuals, particularly homosexual men. Tumors appear multifocally and are characterized by endothelial cell activation and proliferation, as well as by inflammatory infiltrate, particularly in early stages. In progressed stages, the so-called

KS spindle cells, which are regarded as the tumor cells of KS, dominate the histological picture [20–22].

The role of EC in the pathogenesis of AIDS is not only restricted to the above mentioned pathological settings. EC from specific anatomical districts may be infected by HIV-1 and act as virus reservoir [23–26] or facilitate infection spreading in some tissues [27]. Finally, they regulate the passage of infected lymphocytes to lymphoid organs [28–30] and may contribute in metastasis dissemination of AIDS-associated lymphomas [31]. The involvement of EC in AIDS pathogenesis may be propelled by HIV-1 infection, by virus products or by infective agents responsible of opportunistic infections, that may alter EC behavior.

This review will update the most important aspects of EC interactions with HIV-1 and will emphasize the role of the activation of these cells in the pathogenesis of AIDS associated diseases, in particular of KS.

2. Restricted susceptibility of EC to HIV-1 infection

Cellular entry of HIV-1 requires binding to both CD4 and to one of the seven transmembrane G-protein-coupled chemokine receptors, which act as co-receptors. HIV-1 strains have earlier been characterized by their ability to produce syncytia following infection of neoplastic cell lines (viral phenotype). Syncytium inducing viruses are frequently found in progressive or late-stage HIV disease while nonsyncytium-inducing viruses are present throughout disease. HIV-1 can also be classified by its ability to infect primary macrophages and CD4+ T cell lines (cell tropism). All HIV-1 isolates can replicate in primary T cells. However, syncytium-inducing isolates that have adapted to T cell lines cannot replicate in macrophages, whereas some, but not all, primary non-syncytium inducing and syncytium inducing isolates can infect macrophages. This simple paradigm is complicated by the presence of dual tropic HIV-1 strains containing both syncytium inducing and non-syncytium inducing constituents capable of infecting both T cell lines and primary macrophages. With the discovery of the chemokine receptor family as HIV-1 entry co-receptors, HIV-1 strains can also be classified by co-receptor utilization. Strictly non-syncytium inducing (T cell tropism) viruses primarily utilize CC receptor (R) 5, whereas strictly syncytium-inducing viruses (macrophage tropism) primarily utilize the chemokine receptor CXCR4. However, most primary syncytium inducing isolates use CXCR4 in conjunction with CCR5. Not all non syncytium-inducing isolates are capable of infecting macrophages and not all CCR5-using isolates can infect macrophages. Furthermore, some HIV-1 strains can infect the cells through a CD4-independent way [32,33].

EC may be infected by lentiviruses but there are differences related to EC heterogeneity. EC from large vessel do not express CD4 molecule but may be infected by different strains of HIV-1 and HIV-2 [26,34,35] through CXCR4 [36] that is constitutively expressed on this cell type [37]. The stromal-derived factor-1β, the ligand of CXCR4, may down-modulates its receptor and reduces the virus entry [38]. In this type of EC mature virus production is evident during the first days after infection but then declines to undetectable levels, both in the supernatants and in the cell. However, in co-culture experiments with infected EC from large vessels and CD4 positive lymphoid cells or mononuclear cells, it has been demonstrated a productive HIV-1 infection with syncytia formation of lymphoid cells [35]. This rescue activity of T cells and monocytes is enhanced by interferon (IFN)-γ and by RANTES through the up-regulation of intercellular adhesion molecule-1 (ICAM-1) on EC surface. This adhesion molecule seems to be pivotal in rescue mechanism, as inferred by the inhibitory role of antibodies anti-ICAM-1 in the establishment of a productive infection in the co-culture [39,40]. It is possible that ICAM-1 triggered by LFA-1 expressed on adhering lymphomononuclear cells elicits positive signals for virus replication. Similarly, the treatment of EC from large vessels with inflammatory cytokine [(i.e. tumor necrosis factor-α (TNF-α) or interleukin (IL)-1] allows virus replication [41]. These data suggest that EC from large vessels are a

reservoir of HIV-1 that is activated when EC are perturbed.

Microvascular EC from brain, kidney glomeruli, hepatic sinusoid and bone marrow may be infected by HIV-1 and let virus replication without cytolysis [23–26,41]).

The infection of brain EC have been extensively studied for its relevance in neurological diseases associated to HIV-1 infection and for therapeutic implications because the central nervous system also may act as a persistent reservoir of virus since antiviral agents penetrate the blood-brain barrier poorly. T-cell tropic but not brain-derived macrophage tropic HIV strains selectively infect in vitro brain endothelium suggesting that T-cell tropism is important for HIV entry through the blood-brain barrier [23,27] and for in vivo infection of EC in central nervous system [42–44]. A detailed analysis performed by Moses and coworkers excluded a role for CD4 and galactosylcerebroside in EC infection and may suggest the existence of strains with a specific tropism for brain EC besides those with a double specificity for EC and macrophages or CD positive cells [23,24]. The viral sequences responsible for EC infection map to a region that encompasses the C1 region of *env* and includes overlapping reading frame for the accessory genes *vpr*, *vpu*, *tat*, and *rev*.

A role for the gp120 glycoprotein viral coat has also been suggested as mediator the adsorptive endocytosis of the virus in brain EC [45] by interaction with a CCR5 or CXCR4 [46]. However, it has not been exactly established the receptor used by HIV-1 to infect cerebral EC. EC have CXCR4 receptor [37] and express CCR5 at site of inflammatory injury [47] and both may sustain CD4-independent cell infection [32,48–50]. Rhesus brain EC are preferentially infected by macrophage-tropic simian immunodeficiency virus (SIV) [51,52], which uses as primary receptor CCR5 [49]. It is intriguing that circulating mononuclear cells transfer CCR5 to EC during transendothelial migration and enhance the susceptibility of EC to a productive infection during chronic inflammation [53]. Additional orphan chemokine receptors that can mediate HIV-1 or SIV cell entry could be also involved in EC infection.

The susceptibility of brain EC to a productive HIV infection may be relevant for neurological problems associated to AIDS. Through infected EC, HIV-1 may easily spread to the brain and manifests its neuropathogenicity. Alternatively the productive infection may alter features of blood-brain barrier and thus favor the infiltration of infected cells or of HIV-1 itself [54].

3. Direct effect of HIV-1 on EC behavior

On the light of low and discrete susceptibility of EC to be infected by lentiviruses, there are few data on the their direct effects. Infected EC show a selective impairment in the storage and/or excretion of molecules such as endothelin-1 and Von Willebrand factor [55]. The in vitro cytotoxicity is negligible, but EC apoptosis occurs in small and medium-sized blood vessels in the brain of AIDS patients [56], an abnormality that might disrupt the blood-brain barrier [11]. It is likely that EC apoptosis may be induced by soluble stimuli released by immunocompetent and stromal cells or by viral proteins. Among them, HIV-1 gp120 is able to activate an apoptotic program in EC isolated from large vessels [46] and changes the function barrier of microvascular EC monolayer by increasing vasopermeability to middle-sized proteins [57].

4. Indirect effect of HIV-1 on EC behavior: the role of Tat

Tat is a transcriptional activator of viral gene expression produced early after infection and essential, for virus replication. The protein is composed of 86–104 aminoacids (according to viral isolate) encoded by two exons. In the portion encoded by the first exon (72 amino acids), four distinct regions can be recognized (N-terminal, cysteine rich, core and basic). The second exon encodes the C-terminal region containing a RGD sequence [58]. During acute infection of T cells by HIV-1, Tat is released from the cells in an active form [59,60] and via a leaderless secretory pathway that is specific and resembles that of IL-1 and

fibroblast growth factors (FGF) [60]. In addition to its effect on paracrine and autocrine virus replication, it possesses other activities on cell functions. Tat easily enters different cell types contributing to the transactivation of HIV-1 long terminal repeat promoter in latently infected cells [61,62]. Alternatively, it acts as soluble mediator affecting physiologic functions of cells including T and B cells, monocytes, chondrocytes and neurons [63–68]. However, one of the most relevant targets for Tat is the vascular system, where it activates a pro-inflammatory and angiogenic program. Tat can up-regulate the expression of EC adhesion molecules [69,70] and induces EC to proliferate, release proteolytic enzymes [71–79]. These effects are potentiated by cell priming with inflammatory cytokines [71,72,80]. Finally, Tat induces EC adhesion to proteins of extracellular matrix and stimulates EC to undergo in vitro morphogenesis [73,78]. The counter part of these in vitro effects of Tat on EC is its in vivo angiogenic activity [73–76,81].

The molecular mechanisms leading to EC activation by Tat had been extensively studied. Inflammatory cytokines, and in particular IFN-γ, render EC in vitro more responsiveness to Tat by inducing the expression of α5β1 and αvβ3 integrins [72,78,82] that is a feature of activated, angiogenic EC in vivo [83]. Through its RGD sequence, Tat binds integrins [84,85] that signal inside the cells [86], and induces the phosphorylation of p125 focal adhesion kinase (B. Ensoli, unpublished data). Furthermore, inflammatory cytokines induce basic FGF expression that, in turn, induces the same integrins [80,82] and it is required for Tat-angiogenic effect [74]. The basic domain of Tat may compete with basic FGF for binding to heparin sulfate proteoglycans of the cell surface and extracellular matrix [87] and render the growth factor able to activate EC [79].

Additionally, Tat binds to and activates the tyrosine kinase receptor encoded by KDR [vascular endothelial growth factor (VEGF) receptor (R) 2] in EC and Kaposi's cells [76,81,88–91]. The natural ligands of VEGFR2 belong to the VEGF family [92] and a neutralizing antibody anti-VEGFR2 or cross-desensitization between Tat and VEGF-A_{165} block the activation of the receptor as well as the migration of EC triggered by Tat. These results are also supported by recent data showing that Tat has vasopermeabilizing effect by a direct action on EC [93,94], as demonstrated earlier for VEGF-A [92]. These effects are blocked by functional inactivation of VEGFR-2 [93].

To identify functionally important domains responsible for the activation of the angiogenic program in vascular EC, a structure-activity relationship study of Tat protein has been performed [81]. Tat binds EC with high ($K_d \approx 10–30$ pM) and low affinity ($K_d \approx 1–2$ nM). The basic and the cysteine-rich domain mediate the former. Tat basic domain is crucial for the binding and the activation of VEGFR-2. The relevance of the positively charged aminoacids in performing these functions is consistent with the observation that the charged residues R82, K84, H86 of VEGF-A are important for VEGFR-2 recognition [95]. Since it has been suggested that Tat forms a dimer bridging cysteine-rich regions from each monomer [61] and that two cysteine residues are pivotal for the VEGF-A dimerization and the subsequent binding to and activation of endothelium [96], it could be hypothesized that the active form of Tat on endothelium has a dimeric structure. The relevance of this domain has been also emphasized by Albini and co-workers who demonstrated its role in the migration of monocytes [97] another Tat function that requires VEGFR1 [64,98]. The C-terminus region of Tat containing RGD sequence is involved in the low affinity binding sites. Mutants in this sequence or Tat_{72} that lacks the product of exon 2, have reduced biological activity in vitro and in vivo, which is consistent with the earlier reports showing that integrins αvβ3 and αvβ5 participate in Tat-induced activation of EC [72,78,79]. All together, these data suggest that regions encoded by the first exon of *tat* are necessary and sufficient for activation of VEGFR-2. However, the C-terminal region, through RGD-mediated integrin engagement, is indispensable for a full activation of an in vitro and in vivo angiogenic program. These results are not contradictory and it is possible that Tat stimulates EC by an independent activation of integrin system and VEGFR-2 that achieve a synergy in term of

biological functions. However, integrins and in particular αvβ3, show a regulatory activity on tyrosine kinase receptors [89,99,100]. We have recently shown that integrin αvβ3 is associated to VEGF-A-stimulated VEGFR-2 and that a monoclonal antibody against β3 integrin inhibits the activation of the receptor [89]. The blocking effects of neutralizing antibody anti-αvβ3 or anti-VEGFR-2 [75,76,79,89] and the mapping studies performed with mutants [81] or with overlapping Tat peptides [75,76,79] suggest that Tat is a unique example of molecule which turns on intracellular signals by a direct activation of both receptor and integrin systems. Furthermore, beside to have a direct role in integrin activation, the C-terminus region of Tat could regulate VEGFR-2 through the presence of other determinants relevant for the engagement of neuropilin-1, a co-receptor of this tyrosine kinase receptor [101,102].

5. Regulation of leukocytes diapedesis by EC

The changes in soluble mediator networks characterize the early and late events of HIV-1 infection [103,104] and the abnormal increased of circulating inflammatory cytokines [TNF-α, IL-1, IL-8, monocyte chemotactic peptide (MCP) -1] renders EC more adhesive and favors the diapedesis of circulating cells [17]. Furthermore, the presence of HIV in lymphomononuclear cells, the viral proteins Tat, Nef and gp120 and the unbalance of cytokine system alter the expression of adhesion molecules (CD11a/CD18, CD49d/CD29, CD58, CD62L, CD44) on cell membrane and modify the transmigration properties of circulating cells across the vessel walls [28,70,106–111].

Furthermore, Tat also may modify the adhesion molecule profile in EC. As mentioned before, Tat induces the expression of E-selectin, up-regulates ICAM-1 and VCAM-1 in macrovascular and microvascular EC [69,70,112,113] and supports leukocyte adhesion. Furthermore, it stimulates transcription and release of IL-8 [112] and MCP-1 [93] that are powerful leukocytes chemoattractants. In vivo, the effect of Tat on leukocyte recruitment is preceded by an increased in vascular permeability, which is caused by the synthesis of platelet-activating factor in EC and may facilitate cell transmigration [93].

The role of EC in the control of leukocyte diapedesis is crucial in the development of AIDS. During transmigration, the engagement of the integrin system on infected lymphomononuclear cells and the production of inflammatory cytokines by EC result in viral replication in lymphocytes and monocytes [114,115], thus influencing the passage of HIV-1 from latency to productive replication and enhancing virus spreading. Furthermore, abnormal leukocyte traffic in is instrumental to progressive injury of lymphoid tissue and impairment of immune response. Similarly, leukocyte infiltration and abnormal cytokine response are typical features of HIV-1-associated tissue pathologies, including tumors, opportunistic infections and central nervous system degeneration [103–105,116].

6. Kaposi's sarcoma

KS is a multifocal proliferative disease of vascular origin found in four clinic-epidemiological forms. AIDS-associated KS (AIDS-KS) is the most frequent tumor of HIV-l infected homo-bisexual men and is the most aggressive form of KS [21]; 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 [117]; classical KS (CKS) occurs in elderly men of the Eastern–Mediterranean area and is a milder form of the disease [118]; post-transplant KS (PKS) occurs in transplanted individual after therapy with cyclosporin and corticosteroids [119]. 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) histopathology of the lesions; (iii) high levels of the same inflammatory cytokines, angiogenic molecules and growth factors in the lesions; and (iv) 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 inflammatory cytokines 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. 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 cases of tumor regression support the hypothesis of the reactive nature of KS [120]. However, recent evidence also suggests that in later stages of development, reactive KS lesions may transform to a true sarcoma [121,122].

KS lesions are characterized by multiple patch, plaque or nodular lesions particularly on the skin of the extremities but often involving also the mucosae 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 [22,123].

Histologically, early lesions are characterized by an inflammatory-granulation type reaction with activated proliferating EC, 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) 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 [124,125].

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 $CD8^+$ cells but also containing $CD4^+$ cells, numerous monocyte-macrophages ($CD4^+$, $CD14^+$, $CD68^+$, $CD45^+$, PAM-1^+) often with a spindle-like morphology and a subendothelial localization, dendritic cells (FX111a1) and few B cells ($CD19^+$, $CD20^+$ or $CD30^+$) [126–129]. 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 [130]. Therefore, KS cells produce chemokines which are able to recruit inflammatory cells [131–134]. As discussed later, these inflammatory cells, mostly $CD8^+$ cells and monocytes-macrophages, produce a variety of cytokines and in particular IFN-γ, that function in a synergistic fashion to activate EC, to induce the production of angiogenic factors and a further recruitment of T cells and monocytes.

The nature of KS 'spindle' cells has been debated for a long time. Recent studies indicate that these cells are a heterogeneous population with three distinct phenotypes, one reminiscent of activated vascular and lymphatic EC, the other one of macrophagic and dendritic cells, the last of characterized by the presence of mixed markers of macrophage and EC [126–128,135,144].

The reactive or hyperplastic KS cells 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 [121,122,136–138]. These lesions regress as early KS lesions can regress in humans 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 KS cells are 'trans-differentiated' cells and that in late stage, they may transform [121,122,139].

Two transformed cell lines have been established from KS lesions that are able to give tumors in SCID but not in nude mice [121,122,140] 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 [141]. 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 [142] suggesting that tumor transformation may occur in some cases of advanced KS patients that are severely immunocompromised 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 [143].

7. The role of $CD8^+$ cells and inflammatory cytokines in Kaposi's sarcoma

Clinical observations suggest a role for a $CD8^+$ cell activation and production of inflammatory cytokines of the Th-1 type (IFN-γ and IL-2) in KS development [22]. Recent evidence indicates that this is the case. In fact, activated peripheral blood mononuclear cells from both AIDS-KS and CKS patients produce high levels of IFN-γ and little or no IL-4 as compared with patients without KS but with other dermatological disorders [144]. $CD8^+$ cell and macrophage activation with cytokine production is also found in KS lesions from the same patients [126,144]. Thus, immunoactivation is a trait of individuals developing KS and production of cytokine including IFN-γ, IL-1, TNF-α appears to be key to KS development. In fact, the administration of IFN-γ, IL-2 or TNF-α to KS patients leads to disease progression or to KS development [145,146].

A variety of inflammatory cytokines is expressed in lesions from all forms of KS. These include IFN- γ, TNF-α, IL-1, IL-6, granulocyte/macrophages colony stimulating factor, oncostatin-M [126,144,147–149]. They are all produced by infiltrating leukocytes. This cytokine production is associated with vessel activation ($ICAM\text{-}I^+$, $ELAM\text{-}I^+$, $VCAM\text{-}1^+$, DR^+, $CD40^+$, up-regulation of α5β1 and αvβ3 integrins) [74,126,127] and increased vascular adhesion of inflammatory cells [17].

Cytokines produced by activated T cells induce the long-term growth of cultivated KS cells, [150,151] and increase the in vivo angiogenic potential of KS cells [80]. Among them, oncostatin-M and IL-6, in particular, have been found to be strong KS cell growth factor [149,207,208]. However, it is clear that the effect of cytokines on KS cell growth is mediated by a synergistic stimulatory effect on production of angiogenic inducers. Besides basic FGF [74], inflammatory cytokines produced in KS lesions also induce cultured EC and KS cells to produce angiogenic molecules, and other cytokines and chemokines with effects on cell recruitment, growth, angiogenesis and lesion formation (discussed below). In addition, upon exposure to inflammatory cytokine EC become angiogenic in nude mice and induce formation of KS-like lesions as KS cells do [71,80]. Similarly, inoculation of inflammatory cytokines induces KS-like angiogenic lesions in mice [79], indicating that they can trigger a cascade of events leading to lesion formation.

IFN-γ appears to be the major mediator responsible for this cascade, although IL-1 and TNF-α contribute in these effects in a synergistic fashion [71,80]. In addition, IFN-γ up-regulates CD40 expression in cultured KS cells [152] and supports the progression of the lesion by its anti-apoptotic and pro-angiogenic effects probably by induction of the expression of the bcl-2 proto-oncogene [153,154].

Altogether, these results indicate that the cytokines produced in KS lesions are capable of triggering a cascade of events leading to lesion formation and to maintenance and progression of KS.

8. The role of angiogenic inducers in Kaposi's sarcoma

The first experimental evidence that angiogenic factors are involved in KS lesion formation was provided by studies indicating the capability of KS cells to induce angiogenesis in the chorioallantoic membrane assay and highly angiogenic KS-like lesions after inoculation of the cells in nude mice [138,151,74]. These KS-like lesions are of mouse cell origin suggesting that these cells are able to recruit normal vascular cells, a phe-

nomenon also observed in mice injected with human cells carrying mT oncogene of polyoma virus [156]. Lesions regress in time and are mediated by specific angiogenic factors produced by the cells. In particular, basic FGF is a key mediator of lesion formation. Inoculation of basic FGF in nude mice results in the formation of KS-like lesions [74]. In addition to its paracrine activity, basic FGF has autocrine activity in KS development because it stimulates proliferation KS cells [80,157]. Most importantly, both basic FGF mRNA [158] and protein [74] are highly increased in tissue sections of KS primary lesions and in KS cells. This indicates that basic FGF regulates angiogenesis and KS growth in humans and in urine models.

However, neutralizing anti-basic FGF antibodies or antisense oligodeoxynucleotides do not totally block lesion formation after inoculation of KS cells in mice [155] and injection of basic FGF alone does not induce the edema characteristic of KS [74]. This suggests involvement of other factors. In fact, VEGF-A, is expressed as the two secreted forms (VEGF-A_{121}, VEGF-A_{165}) in both KS lesions and in cultured KS cells [157,159,160] and its plasmatic levels are increased in patients affected by KS [161,162]. VEGF-A synergizes with basic FGF in inducing endothelial cell growth and angiogenesis as demonstrated by in vitro and mice studies [157,159]. VEGF-A induces the proliferation and the migration of KS cells [160,163] though activation of VEGFR2 and involvement of c-src and a specific adhesion focal tyrosine kinase [164,165]. The herpes virus HHV-8, a transmissible agent bas been postulated as the causal agent of KS (see below) and it has been recently shown that it increases the transcription of VEGF-A and VEGFR-2 [166,167]. Two other members of VEGF family, VEGF-C, which is expressed in KS lesion [168], and VEGF-D are able to activate the migration of KS by activating VEGFR1 and VEGFR2 [136,169].

Angiopoietins, which act through another tyrosine kinase receptor, Tie-2, are expressed in KS lesions [170] and may be important in the maturation of nascent vessel. In fact, these molecule do not show any proliferative activity but induce cells carrying the specific receptor to release molecules that recruit pericytes and smooth muscle cells around nascent vessels [171].

Another angiogenic molecule found in KS is hepatocyte growth factor (HGF) [90,172]. HGF induces EC to acquire spindle morphology and stimulates proliferation of cultured KS spindle cells. Moreover, HGF and its cognate receptor, the c-*met* protein, are expressed in human KS lesions, suggesting that it may play a role in KS development.

Platelet derived growth factor-B (PDGF) is another potent paracrine-acting mitogen for cultured KS cells that is expressed in vivo by subpopulations of cells that are intermingled with the spindle cells [173]. KS cells express PDGF β-receptor, suggesting that PDGF-B may activate the proliferation of KS cells by paracrine mechanisms. In addition, PDGF-B may have angiogenic activity suggesting that it may also contribute in the angiogenesis found in KS [173–177].

Platelet-activating factor is an autacoid molecule, which activates the migration of KS in autocrine manner [178,179] and has angiogenic activity [180].

Altogether these data indicate that a network of angiogenic factors and spindle cell growth factors 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 cytokines discussed above can explain the mixed cellularity and the angiogenesis of KS lesions in the context of a cytokine-mediated reactive process.

9. The role of human herpes virus 8 and of HIV-1 Tat in Kaposi's sarcoma

Although a transmissible agent bas been postulated as the causal agent of KS and several viruses and other agents have been suggested [22], none has been confirmed. Recently a new herpesvirus termed human Herpesvirus 8 (HHV-8), that is closely related to Epstein-Barr-virus and herpesvirus saimiri, has been identified and shown to be present in all epidemiological forms of KS [181–183]. Since HHV-8 seroprevalence is low in

areas at low incidence of KS and its detection can precede the onset of KS [184–186], these results suggest that HHV-8 is key to KS development but it requires additional factors to exert its effects in KS pathogenesis.

At the lesion level, HHV-8 is present in endothelial and spindle cells mostly in a latent form, [187,188] whereas mononuclear cells including monocytes-macrophages are lytically infected [189] and may support virus production and spread to other cell types.

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 and the kaposin gene (expressed in lytic and latent infection) of HHV-8, whereas in early KS lesions this antigen 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 with late stage lesions [187,188].

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), viral FLICE-inhibitory protein (vFLIP) and potentially transforming genes like v-bcl-2 and v-cyclin D [190–193]. However, most of these genes are expressed during lytic infection and not in latently infected KS spindle cells and only v-cyclin D and vFLIP expression can be detected by in situ hybridization in numerous KS spindle cells of late, but not early nodular KS lesions [194]. It is intriguing to remember that vFLIP induces a protection of virus-infected cells against death-receptor-induced apoptosis and may lead to higher virus production and contributes to the persistence and oncogenicity of HHV-8 [195]. So, 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.

In addition to these genes, HHV-8 encodes three chemokine ligands with interesting functional characteristics and two of them, vMIP-I and -II, can promote angiogenesis [196]. Transgenic mice expressing the HHV8-encoded viral G protein–coupled receptor (GPCR, a homologue of CXCR1and CXCR2) within hematopoietic cells develop angioproliferative lesions in multiple organs that morphologically resemble KS lesions. These lesions are characterized by a spectrum of changes ranging from erythematous maculae to vascular tumors, by the presence of spindle and inflammatory cells, and by expression of VEGF-A [197]. This viral protein is a constitutively signalling receptor that can bind chemokines from the CXC and the CC families, but does not require ligand for its activation [198]. It was shown that signalling by HHV-8 leads to the up-regulation of expression of VEGF-A, thereby inducing angiogenesis via a paracrine mechanism(s). In KS lesions, there is a small subset of cells expressing lytic genes that appear to be productively infected with HHV-8 [199] and GPCR is expressed by these scattered cells. Therefore, it can be argued that in KS lesions the expression of this viral receptor by a subset of infected cells could have a major contribution to HHV8-induced angiogenesis. This finding suggests that HHV8 GPCR is a critical viral gene involved in the pathogenesis of KS, and points to the dramatic effect of paracrine stimulation of angiogenesis mediated by VEGF-A secretion in KS pathogenesis.

On the other hand, the higher viral load found in KS patients and in late-nodular lesions suggests that individuals at risk of KS offer better conditions to virus growth and spread in the body. The same cytokine 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 [200], likely promoting HHV8 transmission to other cell types.

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-I itself may play a role in KS development.

The earliest evidences to support the role of Tat in AIDS-KS come from Tat transgenic mice that develop KS-like lesions [201,202]. Although Tat expression in both male and female mice was comparable, lesions developed only in male mice, mimicking the male predominance. More recently, another study supports the idea that this molecule accounts for the aggressiveness of KS in AIDS. In these animals the growth of KS cells was six fold higher than that in the non-transgenic mice [203].

In vitro studies indicate the specific role of this viral protein in activation of KS cells. Tat is capable of inducing the growth, migration, adhesion and invasion of KS cells [72–74]. KS cells express both VEGFR2 [88,90,91,136] and $\alpha 5\beta 1$ and $\alpha v\beta 3$ integrins [78,79,82] that are the receptors for Tat mediating EC activation. Furthermore it promotes the synthesis by mesenchymal cells [204] of basic FGF and has synergistic activities with this growth factor in activation of KS cells [74].

Tat has also been shown to activate the adhesion and transmigration across capillaries of monocytes–macrophages that characterize KS lesions and contribute in production of angiogenic molecules and KS growth factors (see above) [64,67,97,109].

Extracellular Tat is detectable in AIDS-KS lesions and co-stains with these receptors on spindle cells and activated vessels, 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 [74].

Furthermore, the presence of detectable extracellular Tat in sera from AIDS patients [205], as well as the presence of antibodies anti-Tat in AIDS-KS patients, which recognize different epitopes as compared with those in AIDS patients [206] support the hypothesis of its role as a progression factor in AIDS-KS.

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