cleosomes impose on transcription (see the figure). The large RNAPII complex also has to extend through DNA that is wrapped around the surface of histone octamers. Consequently, nucleosomes tend to impede the passage of polymerases and can result in a stalled polymerase. Earlier work by Orphanides and colleagues (*5*) suggests that transcription elongation through nucleosomes is facilitated by a distinct accessory complex, designated FACT. FACT has only been biochemically defined and, like RSF, has a simple polypeptide composition of two subunits (p140 and p80). FACT does not stimulate transcription initiation and does not require a promoter-bound transcription activator. The only prerequisite for FACT function is a promoter-remodeled template, suggesting that activities like those of RSF have to precede that of FACT. FACT does not fit the pattern of a conventional remodeling activity because ATP hydrolysis is not necessary for FACT-mediated elongation. This complex is also unlikely to function as a conventional elongation factor like TFIIF or TFIIS because neither activity is able to substitute for FACT (*5, 11*).

The report by LeRoy *et al.* illustrates one potential combination of activities (RSF and FACT) that can overcome multistep nucleosome-mediated inhibition of transcription (see the figure). In these experiments RSF remodels the promoter in the presence of GAL4-VP16 and allows the formation of transcription complexes and the initiation of transcription while FACT facilitates productive elongation through downstream nucleosomes. Although LeRoy *et al.* have used a highly purified system, it is formally possible that other activities might have contributed to the transcription activity. In particular, the histones assembled on the template likely represent a combination of endogenous *Drosophila* embryo histones (from the S-190 assembly extract used) and exogenously supplemented human histones. The acetylation state of these histones might have contributed to the active fraction of the templates (*12*).

It will be interesting to see what other chromatin-remodeling complexes will functionally substitute for RSF or FACT in chromatin transcription. LeRoy *et al.* suggest that the homologous *Drosophila* ISWI complexes are likely to have this property. Moreover, the larger SWI/SNF or RSC complexes will likely perform a similar function, perhaps in more specialized circumstances; for example, SWI/SNF participates in chromatin remodeling at the SUC2 promoter in yeast (*13, 14*) and in tissue-specific transcription of the human β-globin gene (*15*). SWI/SNF has also been implicated in contributing to the process of transcriptional elongation (*16, 17*). The situation in vivo is further complicated by the possibility that quite different chromatin-modifying complexes (such as SWI/SNF and the SAGA histone acetyltransferase complex) may be functionally redundant (*18*). Thus, there may be multiple combinations of activities that might be called on by distinct promoters to solve the nucleosome problem during transcription.

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PERSPECTIVES: BIOMEDICINE

The Enigmas of Kaposi's Sarcoma

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No example illustrates the difficulties in understanding tumor biology better than Kaposi's sarcoma (KS). The etiology of this tumor is not easy to define; a metastatic malignancy cannot easily be distinguished from a nonmalignant growth that occurs in multiple sites; and many distinct factors contribute to the pathogenesis of KS.

There are four distinct epidemiological forms of KS. Are these actually the same disease, or are they similar only because the same kinds of cells are involved, as in leukemias, lymphomas, and lung cancers? The first ("classical") form of KS occurs in older males of mainly Mediterranean or Eastern European Jewish backgrounds and has no known contributing environmental factor. A second form, found in parts of equatorial Africa, occurs in all age groups and also has no known precipitating environmental factor. Neither is typically associated with immune deficiency. In contrast, the remaining two types of KS—those associated with organ transplants and with human immunodeficiency virus–type 1 (HIV-1)—are accompanied by immune impairment. Males are predominately afflicted in all forms.

A second problem is the elusive nature of the tumor cell. Many cells in the lesion are clearly normal cells that have infiltrated the tumor, such as leukocytes. The predominant cell in the tumor is a spindle-shaped cell (SC), which is accompanied by abnormal blood vessel development and leakage of blood (see figure). It is reasonable to call the SC the "tumor cell," but there is no direct evidence that this cell is an autonomously growing neoplastic cell rather than a hyperproliferating but otherwise normal cell (hyperplasia) (*1*). Moreover, although most SCs are of endothelial cell origin, there is evidence that some of them arise from other lineages such as macrophages and fibroblasts (*2*). This makes it very likely that only some (if any) SCs are neoplastic because neoplastic cells are usually of one lineage. Some inflammatory cytokines [for example, interferon γ (IFN-γ), which is known to be increased after HIV-1 infection (*3*)] can induce a spindle-like alteration in the shape of endothelial cells and macrophages (*4*). It is therefore an oversimplification to infer that SCs are neoplastic or descended from a single transformed cell clone.

The corollary of this problem raises the third issue: Are any of the cells in the KS lesion neoplastic, or are they all the result of a chronic inflammatory response (*1, 5*)? That is, is KS a malignancy or is it a proliferative inflammatory response, or both? Several lines of evidence indicate that most or all KS proliferative cells are not in fact neoplastic: the three histological features of KS (angiogenesis, inflammation, and proliferation); the absence of a histologically discernible neoplastic cell; the sometimes spontaneous regression of KS; the usual lack of chromosomal abnormalities; the appearance of KS lesions in mul-

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tiple body parts at a similar time; and the equivocal and variable clonality [monoclonal lesions in some cases, oligo- or even polyclonality in others (*6*)]. So is KS really a malignancy?

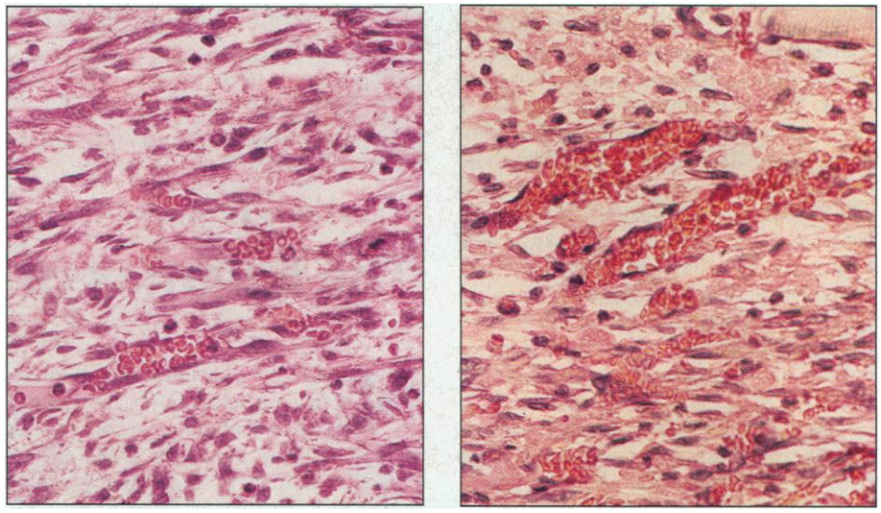
The Viruses and Etio-Pathogenesis

One factor ties together the four epidemiological forms of KS—the novel herpes virus KSHV (or HHV-8) (*7*), invariably found in KS tissues. If, as evidence suggests, HHV-8 is not ubiquitous, then its consistent presence in KS must indicate a key etiological role, which in turn would imply that the four forms of KS are indeed one, but with different (but unknown) augmenting cofactors. It is also clear, however, that in itself HHV-8 is a very low risk factor for KS development. To illustrate: Most reports suggest a 2 to 10% global prevalence rate for HHV-8, with much higher rates in some areas. Assuming a 5% prevalence of HHV-8 in the United States and a 1970s baseline incidence of KS in men in the United States (about 0.3 cases per 100,000 men), the HHV-8 rate would be one case of KS in every 17,000 HHV-8 infections. The opposite trend is true for HIV-1, which is clearly not needed to cause KS, in that three of the four epidemiological forms of KS occur without HIV-1. Nonetheless, HIV-1 infection is associated with an enormous increase (by a factor of 20,000 to 50,000) in the incidence of KS in the presence of HHV-8. Indeed, a recent report shows that HHV-8 and HIV-2 infections in Gambia are common, whereas KS is rare unless accompanied specifically by HIV-1 infection (*8*).

HIV-1

What is the role of HIV-1 in KS? Generally, it is assumed that HIV-1 infection simply promotes HHV-8 replication indirectly by impairing the immunity of the host. We postulate a more specific role: KS begins as microvascular inflammatory lesions, fostered by a different set of environmental factors in each of the epidemiological forms. One of these sets of factors is aberrant cytokine production (*1, 5, 9, 10*); in HIV-1–associated KS, this includes a marked increase of inflammatory cytokines, notably IFN-γ, tumor necrosis factor–α (TNF-α), and the interleukins IL-1 and IL-6 (*1, 3, 5, 10*), augmented by different activities of HIV-1 Tat (*1, 11*). Some of these cytokines promote activation and growth of endothelial cells, the expression of adhesion molecules and integrins, the production and release of angiogenic molecules [basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF)] by these endothelial cells (*11*), and an increase in HHV-8. Tat, essential for HIV replication, is also released from acutely infected T cells (*11*) and taken up by nearby cells (*11, 12*), where it impairs proliferation (in T cells) (*12, 13*) and promotes cytokine dysregulation, adhesion, and growth (in the endothelial cells) (*11*). Thus, in addition to its essential role in HIV replication, Tat is a toxin with much wider effects on uninfected cells.

Tat interacts with endothelial cells via its basic region and more specifically via its RGD motif, which is absent in HIV-2 and in simian immunodeficiency virus (SIV). Interestingly, infection with HIV-2 is much less often associated with KS, and monkeys infected with SIV do not develop KS even when they are immune deficient and also infected with HHV-8–related viruses (*14*).



Spindle-shaped cells of Kaposi's sarcoma. Blood vessels with red blood cells are also present.

HHV-8

HHV-8 is clearly linked to KS (*15*), but what is the role of HHV-8 (KSHV) in KS tumor development? The situation is more complex than is usually appreciated. Although some workers consider HHV-8 to be a highly oncogenic and transforming virus, the evidence is not clearcut. For example, ORF 74, an HHV-8 homolog of a G protein–coupled receptor, is expressed in some KS tissues and induces expression of the angiogenic cytokine VEGF and cell growth (*16, 17*); although these findings are of interest, they cannot be taken as evidence that HHV-8 is oncogenic or is responsible for angiogenesis in KS lesions. Expression of ORF 74 is generally restricted to lytic phase replication and a small minority of cells, and the cells in which it is expressed are probably destined to die, not grow. Furthermore, in some lesions, expression is not detected at all by the reverse transcription polymerase chain reaction (*17*). Expression of VEGF in a tumor composed of many newly forming blood vessels is notable, but because ORF 74 is not expressed in some KS tumors whereas VEGF is regularly found, the origin of VEGF expression must be more complex than simple induction by ORF 74. Indeed, VEGF (and bFGF) production is also stimulated by inflammatory cytokines induced by HIV-1 infection (*18*). In addition, at least some of the few neoplastic cell lines available from KS tumors constitutively produce VEGF, and none carry HHV-8 sequences (*19*).

Some genes of HHV-8 transfected into NIH 3T3 mouse cells produce malignant tumors in nude mice, whereas control cells do not. This result has been used to argue for a direct oncogenic capacity of HHV-8 in patients (*20*). However, this effect can also be produced with genes from other viruses, such as adenoviruses and HHV-6, neither of which have transforming activity for any primary human target cell or are known to cause any tumor. Moreover, there is no conclusive evidence that the spindle cells of KS lesions are neoplastic, or even that most KS is a neoplasia rather than a hyperplastic chronic inflammatory response of mixed cellularity. Thus, the effect on mouse NIH 3T3 cells cannot be taken as conclusive or even strong evidence for a neoplastic transforming effect in vivo.

Recently, the first evidence was presented for in vitro growth promotion of endothelial cells by HHV-8 with implications for neoplastic transformation (*20*). The infected cells were more growth responsive than uninfected cells to high concentrations of VEGF, but they were continuously dependent on VEGF, exhibited unusual re-

quirements for growth on soft agar (supplemented with 40% fetal calf serum and high concentrations of endothelial cell growth supplement), and did not show tumorigenicity in an animal model, leaving serious doubts as to whether these effects can be called transformation, or even immortalization. Nonetheless, this study clearly shows a direct growth-promoting effect of HHV-8 on the main proliferating cells of KS, the endothelial cells.

The same inflammatory cytokines that are increased after HIV-1 infection enhance replication of HHV-8 (*21*). Cells infected with HHV-8 may home to preexisting microinflammatory lesions, perhaps because of ORF 74, the HHV-8 chemokine receptor homolog. In this milieu, HHV-8 may gradually become more abundant, and either through its subsequent infection of endothelial cells or through its own induction of growth-promoting cytokines, it may foster more and sustained proliferation, sometimes culminating in a neoplastic clone. Some clinical results favor this interpretation. Administration of IFN-γ and TNF-α in clinical trials was associated with an explosive growth of KS (*22*). Although inhibitors of HHV-8 (gancyclovir and foscarnet) have been associated with inhibition of KS lesions, the results are inconsistent, and if HHV-8 is a transforming virus, the inhibition of its replication should have no effect on the tumor, or at least not on neoplastic cells. These results would be more consistent with the view that the bulk (or all) of the tumor lesions of KS are not composed of neoplastic cells.

On the other hand, cell lines from three different patients have been established by independent groups [one from a patient with HIV-1–associated KS (*23, 24*), another from a patient who had undergone a renal transplant and had transplant-associated KS (*24*), and recently a third from a patient with classical KS] (*22*). These cells grow permanently, induce sarcomas in nude mice, and exhibit common chromosomal changes (*22, 25*). The similarity of the chromosome abnormalities from the three cell lines suggests that they are a true representation of the malignant KS cell. However, these abnormalities are not detectable in primary tumor biopsies. Is this because neoplastic cells are rare in KS lesions or because only an occasional lesion evolves into a malignancy? Studies with these cells suggest that KS evolves malignant clones in at least some cases, probably during late stages. Other results suggest that any malignant cells are present in small numbers and recruit normal cells, analogous to the Reed-Sternberg cells of Hodgkin's disease (except that in KS the cell is not morphologically identifiable).

These issues are more than academic. If KS is not a malignancy and requires replication of HHV-8 (and HIV-1 in the case of HIV-1–associated KS), future research on therapy would logically include antiviral strategies. If, however, neoplastic cells are invariably a component of a KS lesion, even at the earliest stages of tumor development, our focus should be more on cytostatic or cytotoxic drugs.

It is of considerable interest that none of the three known neoplastic KS cell lines contain HHV-8 sequences, nor do any of the short-term (past two passages) cultures of KS SCs. Does this mean that the putative HHV-8 "transformed" cells immediately die in culture and that the rare obtainable surviving transformed cells (such as the three known cell lines) lose the viral sequences? This is one interpretation, but in my view is unlikely. The other interpretation is that HHV-8 facilitates hyperplasia of some infected endothelial cells, and also of nearby cells, by paracrine action and does not transform its target cell. In this scenario, transformation when it occurs involves a separate lineage; many of the real tumor cells were never infected by HHV-8.

But what of the forms of KS that do not involve HIV-1 infection? Does KS develop in them solely as a result of HHV-8 infection, or are other factors required? In the case of organ transplant–associated KS, the argument that this is the result of the intentional drug-induced immune deficiency seems obvious. However, it could also be because the graft may provoke chronic production of inflammatory cytokines. Cofactors for the classical and African forms of KS are not defined, but as noted earlier, these are not linked to immune deficiency.

Male dominance in KS is another remaining enigma. Explanations for this prevalence usually invoke a greater prevalence of HHV-8 in males, but this is not borne out by current epidemiological studies.

Conclusion

The KS tumor is complex in its cellular composition, origin, epidemiology, and pathogenesis. It begins as a result of different stimuli that promote microvascular inflammation; one major stimulus is HIV-1 infection. Development as a clinical tumor may depend on activation by HHV-8 and by cytokines (and Tat in HIV-1–associated KS) in the inflammatory lesion. HHV-8, in turn, may promote cell growth by augmentation of these and other growth-promoting cytokines. It is likely, then, that KS begins as a hyperplasia and may evolve into malignant clones in some patients, but the neoplastic cells may be a small minority. Thus, KS may initiate as a microenvironmental abnormality with secondary consequences, analogous in some respects to tumors arising from so-called landscape defects. Multiple issues regarding the virus, the epidemiology, and the tumor still remain unsolved. These include the true prevalence of the virus; its routes of transmission; the percentage of spindle-shaped cells infected in the earliest lesions; the paradox of the absence of HHV-8 sequences in growing KS spindle cells in vitro; the development of an animal model; the reasons for the male dominance; the cofactors for African (endemic), classical, and transplant-associated KS; a method for the detection and identification of the neoplastic cells in vivo; and an unambiguous explanation for the marked recent decline in KS despite the continued presence of HHV-8 and of HIV-1 infections (currently attributed to the decrease in HIV-1 viral load as a consequence of aggressive anti-HIV therapy).

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