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TECHNICAL COMMENTS

Herpes-Like Sequences in HIV-Infected and Uninfected Kaposi's Sarcoma Patients

Recently, Yuan Chang *et al.* (*1*) detected the presence of unique DNA sequences in 90% of Kaposi's sarcomas (KS) and 15% of non-KS tissues in HIV-infected individuals. These sequences, amplified by polymerase chain reaction (PCR) procedures and sequenced by Chang *et al.*, share DNA homology with genes of the capsid and tegument proteins of *Herpes saimiri* and Epstein-Barr viruses (*1*). In evaluating the presence of this herpesvirus-like sequence, we have synthesized primers for one of the reported sequences, designated the KS330Bam fragment, and used the PCR procedure to examine 13 KS biopsies, 12 corresponding normal tissues, 7 KS-derived cell lines, and peripheral blood mononuclear cells (PBMC) from 30 subjects (*2*).

Our studies confirm those of Chang *et al.* (*1*) and indicate that the herpesvirus-like sequence can be found in all 13 KS biopsies studied, including one from an individual not infected with human immunodeficiency virus (HIV) (Table 1). All 13 biopsy donors were homosexual men living in the United States. Corresponding control skin and other nearby tissues from eight of these men were negative for the sequence. In the four exceptions, the presence of KS cells in the control biopsies could not be excluded by histologic examination. In one patient whose KS tumors were positive for the KS330Bam sequence, subsequent biopsies of three resolved KS skin lesions were negative. These results were obtained after the patient had received chemotherapy and the biopsies revealed no histologic evidence of KS.

The SLK cell line, derived from an HIV-negative KS patient (*3*), was negative for the KS330Bam sequence (Fig. 1), as were six cell lines derived in our laboratory from KS tissue (*4*). This herpesvirus-like sequence was also not detected in EBV-carrying cell lines (for example, Raji) nor cells infected with HHV-6 (*5*).

We have detected the KS330Bam sequence as well in the PBMC of 10 KS patients and not in the PBMC of 20 non-KS subjects studied (*6*). Six of the non-KS individuals were HIV-infected, while the others were healthy, HIV-seronegative subjects. Of the 10 KS patients, three were not infected with HIV (Table 1).

With the use of immunomagnetic bead selection (*7*), we have isolated cell subsets from the PBMC and localized the KS330Bam sequence primarily to the $CD19^+$ B cell population (Table 1). Notably, the $CD8^+$ cells were negative. These findings are consistent with the presence of this sequence in some B cell lymphomas (*1*). Finally, in attempts to examine the possible route of transmission of this KS-associated virus, we examined cells and cell-free fluid from saliva and

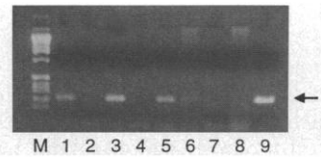

Fig. 1. Analysis of PCR amplified herpesvirus-like sequence. Total DNA from tissue or cells was amplified, and electrophoresis was performed on an agarose gel (*2*). M, DNA molecular weight marker. Lanes 1 and 2, tumor and control tissue from KS+ HIV+ patient; lanes 3 and 4, tumor and control tissue from KS+ HIV− patient; lane 5, KS+ HIV+ PBMC; lane 6, KS+ HIV-PBMC; lane 7, KS-HIV-PBMC; lane 8, SLK line; and lane 9, positive control KS tumor tissue. Arrow indicates the mobility of the 233 base pair portion of the KS330Bam fragment.

Table 1. Prevalence of herpesvirus-like sequence in Kaposi's sarcoma (KS) tissue and blood of KS patients. Studies were conducted as described in text (*2*). Some of the tumor tissues and corresponding controls were provided by the University of California AIDS Specimen Bank under the direction of Dr. John Greenspan. Peripheral blood mononuclear cells from normal subjects came from healthy volunteers in the laboratory or were provided by Irwin Memorial Blood Centers, San Francisco, California.

Specimen	Subject's status		KS330Bam
	KS	HIV	
KS tumor	+	+	12/12
control tissue	+	+	4/11
KS tumor	+	−	1/1
control tissue	+	−	0/1
PBMC	+	+	7/7*
PBMC	+	−	3/3
PBMC	−	+	0/6
PBMC	−	−	0/14
PBMC subsets†	+	+	
$CD8^+$			0/3
$CD19^+$			3/3
Saliva	+	+	
Cells			0/4‡
Fluid			0/2
Semen	+	+	
Cells			0/5‡
Fluid			0/4

*Testing of samples obtained from at least two different visits of three of these patients yielded positive results. Samples from three of these HIV-infected KS patients were used for the subset analysis. †Cell populations with ≥ 90% CD8+ or CD19+ cells. ‡Repeat samples from two patients on separate occasions were evaluated and also gave negative results.

semen of KS patients. Cells were removed by low-speed centrifugation and fluids were ultracentrifuged at 100,000g for 2 hours in a Beckman SW28 rotor. The cells and high speed pellets were resuspended, extracted, and analyzed as described (*2*). The KS330Bam sequence could not be detected in these body fluids (Table 1).

These results, showing the presence of the KS330Bam sequence in KS tissue and PBMC of both HIV-infected and uninfected individuals, provide further evidence of the specific association of a herpesvirus-like sequence with this malignancy. Its absence in the KS-derived cell lines may indicate the lack of cultivation of the KS cell itself, or an indirect effect of a herpes-like virus on endothelial cell proliferation, for example via cytokines as has been proposed (*8*). The preliminary data showing the absence of the KS330Bam sequence in saliva and semen may be a result of sampling error, and the potential route of transmission of this KS-associated agent remains unknown. Further evaluation is also needed to determine the distribution of this KS-associated agent in the population.

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2. The PCR procedure specifically amplifies a 233–base pair portion of the KS330 Barn fragment using primers described by Chang *et al.* (*1*). This 233–base pair fragment is referred to as the KS330Bam sequence in the text, since its detection indicates the presence of the KS330Bam sequence. The conditions for PCR are: 94°C for 1 min (1 cycle); 94°C for 30 s, 58°C for 1 min, 72°C for 90 s (45 cycles); 72°C for 7 min (1 cycle). Each 100-μl reaction contains 0.5 to 0.75 μg of genomic DNA, 30 pmol of each of primer, 2.5 U of Taq polymerase, 200 μM each of deoxynucleotide triphosphate, 10 mM tris-HCl, 1.5 mM $MgCl_2$, and 50 mM KCl (pH, 8.3). Reaction product (10 μl) was electrophoresed on a 1.6% agarose-TBE gel containing ethidium bromide for 45 min (5 V/cm) to verify the presence or absence of the KS330Bam fragment by comparison with the amplified product of a positive control reaction. This control KS330Bam fragment, amplified from genomic DNA extracted from a KS tumor biopsy, was partially sequenced and shown to be identical to the KS330Bam sequence described by Chang *et al.* (*1*). PCR amplification of the human p53 tumor suppressor gene with primers P6-5 and P6-3 (*1*) was used to confirm DNA integrity. Total genomic DNA was isolated from tissue and PBMC by standard techniques.
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Building an Associative Memory Vastly Larger Than the Brain

Leonard M. Adleman proposes using the tools of molecular biology to solve computer science problems such as the directed Hamiltonian path problem (*1*). Richard J. Lipton (*2*) describes an improved biological procedure to solve directly any computational problem in the class NP and suggested approaches to speed up solution of some important practical problems. It is possible, at least in principle, to use these tools to produce an associative, or content addressable, memory of immense capabilities. A content addressable memory is one where a stored word may be retrieved from sufficient, partial, knowledge of its content, rather than needing to know a specific address as in standard computer memories. Content addressable memories are useful in a number of computer contexts and are widely thought to be an important component of human intelligence.

The memory is conceptually simple. It consists of a vessel containing DNA. It would be able to store binary words of a fixed length. One would write such a word in memory by placing in the vessel an appropriate single strand DNA molecule encoding it (*3*). In the simplest approach, two distinct DNA subsequences would be assigned to each component—the first coding for a "one" at that component and the other coding for a "zero." The DNA molecule coding a particular word would be composed by concatenating the appropriate subsequences corresponding to its particular bits, in any order (*4*).

This memory would be "content addressable." Given a "cue" consisting of a subset of the component values, one might retrieve any words in the memory consistent with these values as follows. For each component specified in the cue, one could introduce in turn the complement of the corresponding subsequence of DNA, affixed to a magnetic bead. This compliment would then bond to DNA molecules in the memory having that subsequence, that is, coding for words containing that component value. A similar bonding procedure was used by Adleman in his computation (*1*). These molecules could then be extracted magnetically. After iteratively extracting on each component in the cue, one would retrieve molecules matching the cue exactly and