COMPARATIVE ANALYSIS OF GENOMES OF ONCOGENIC B-LYMPHOTROPIC HERPESVIRUSES REPRODUCING IN CELL LINES OBTAINED FROM DIFFERENT SPECIES OF MONKEYS

V. V. Kakubava, B. A. Lapin, V. V. Timanovskaya, V. Z. Agrba, S. V. Novozhilov, and S. V. Semenova

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Convincing proof has now been obtained of the role of herpesviruses in the induction of some forms of malignant tumors in animals and man. The B-lymphotropic herpesviruses, belonging to the γ-herpesvirus subfamily, have been isolated from many species of old world monkeys and man [3]. Viruses of this group possess transforming activity in vitro, and some members also have oncogenic activity (Epstein-Barr virus — EBV), and baboon herpesvirus (BHV), inducing malignant lymphoma in South-American monkeys [9, 10]. In 1984 a new representative of the group of β-lymphotropic primate herpesviruses, called HVMA, possessing transforming activity not only for lymphocytes of several species of monkeys, but also for rabbits, was isolated in the Research Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR, from a brown macaque. The HVMA also induced a lethal generalized lymphoma of lymphoblastic and prolymphocytic types in rabbits [6]. Later, we obtained lymphoid cell lines producing green monkey herpesvirus (GMHV), from African green monkeys, some strains of which possess oncogenic activity for rabbits [5]. The oncogenic activity of some strains of BHV also has been demonstrated [2].

The aim of the present investigation was a comparative analysis of DNA of B-lymphotropic primate herpesviruses, reproduced in monkey cell lines constituting a collection of lymphoid suspension cultures of primate cells of the Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR.

EXPERIMENTAL METHOD

Cells were cultured by the usual method [1]. Isolation of DNA, the nick-translation reaction, and blot hybridization were also carried out by method described previously [4]. The molecular hybridization reaction in solution was carried out by the following method: DNA preparations were treated with ultrasound 2·30 sec (MSE, ultrasonic disintegrator) and added to an incubation mixture containing, in a volume of 0.11 ml, 0.88 M NaCl + 1 mM HEPES, pH 7.0 (50 μl); fragmented, denatured cell DNA (2 mg/ml) (50 μl), ^{3}H-dCTP-labeled DNA (8000-10,000 cpm) (10 μl). The reaction was carried out at 65°C for 72-96 h. After the end of the hybridization reaction, the heteroduplexes formed were determined after treatment with nuclease S1.

DNA preparations labeled in the nick-translation reaction were used as molecular probes: a) a mixture of cosmid clones of EBV DNA, overlapping the entire virus genome 13; b) BHV DNA and HVMA DNA, isolated from virions and purified in a CsCl density gradient [8].

EXPERIMENTAL RESULTS

To determine the degree of homology of the EBV, BHV, HVMA, and HVGM genomes, the method of molecular hybridization in solution was used. The results of hybridization of the viral DNAs with preparations of total cellular DNA of the corresponding virus-producing lines are given in Table 1.

Research Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR, Sukhumi.
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TABLE 1. Homology of HVMA Genomes

Hybridization zone	Per cent homology of genomes			
	BHV	HVMA	HVGM	EBV
EBV DNA	40	30	25–30	100
HVMA DNA	45	100	—	30
BHV DNA	100	45	—	40

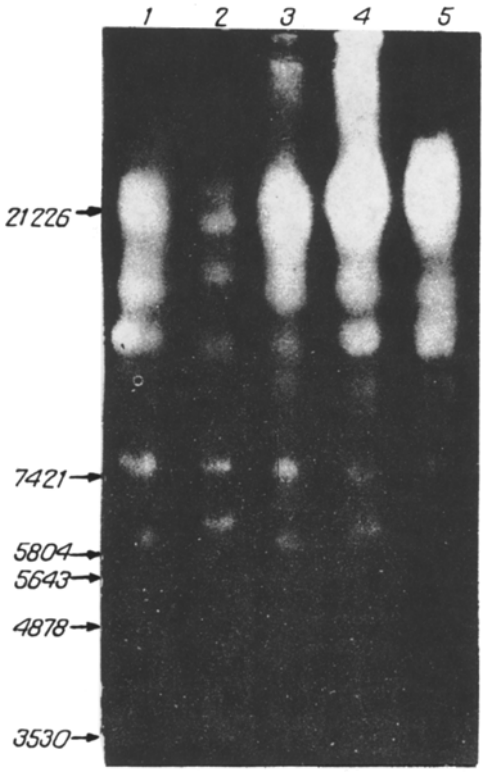

Fig. 1. Blot-hybridization of ^{32}P-DNA from EBV with DNA of cell lines restricted by EcoR1. 1, 2, 3) HVMA-producing macaque cell lines MAL-1, MAL-2, and MAL-3, respectively; 4) BHV-producing baboon cell line LUG-4; 5) human cell line Raji, containing the EBV genome.

The data given in Table 1 are evidence that homology of the genomes of the tested viruses is relatively low (25-45%), and similar to the degree of homology characteristic of other viruses of this group also: herpesviruses of chimpanzees, gorillas, and orangutans [12, 13, 15]. Despite the high content of GC-pairs, characteristic of the DNA of these viruses [11], this relatively low percentage homology of the genomes required milder conditions for the use of the blot-hybridization reaction. The molecular hybridization reaction was therefore carried out at 60-65°C, depending on the viruses studied. Washing the nitrocellulose membrane filters also was carried out under mild conditions: the filters were washed in a solution of 2·SSC, 0.15% SDS, 2 × 30 min at room temperature, and then at 50-55°C in a solution of 0.2·SSC, 0.15% SDS, 4·30 min.

The results of blot-hybridization conducted under these conditions are shown in Figs. 1-3.

Hybridization of EBV DNA with DNA of lymphoid cell lines of macaques, baboons, and man, in which a search was made for viral DNA restricted by EcoR1, is illustrated in Fig. 1. The autoradiograph shows heterogeneity of the test viruses with respect to DNA restriction sites. It is also clear that certain differences exist in restriction sites between different strains of HVMA, reproduced in cell lines MAL-1 and MAL-3 compared with DNA in MAL-2 cells.

Figure 2 gives the results of blot-hybridization of EBV DNA with DNA from various cell lines restricted by Pst 1. Figure 2 also shows both interspecific and intraspecific heterogeneity of the tested herpesviruses with respect to DNA restriction sites. Results were obtained which may be evidence in support of the transforming and oncogenic activity of HVMA DNA. For instance, cell line 20018/MAL-3 was obtained by transformation of peripheral blood lymphocytes of *Papio hamadryas* by HVMA

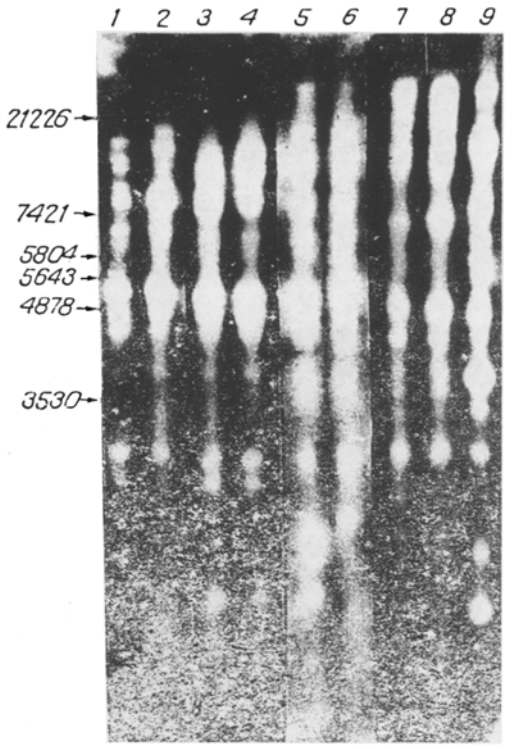

Fig. 2. Blot-hybridization of ^{32}P-DNA of EBV with DNA of cell lines restricted by Pst 1. 1, 2, 3) HVMA-producing macaque cell lines MAL-1, MAL-2, and MAL-3, respectively; 4) cell line 20018/MAL (lymphocytes of *Papio hamadryas*, transformed by HVMA from line MAL-3); 5, 6) cell lines RT-1 and RT-2 obtained from HVMA of induced rabbit tumors; 7, 8) BHV-producing baboon cell lines VPLP-II and LUG-4, respectively; 9) human cell line Raj1, containing the EBV genome.

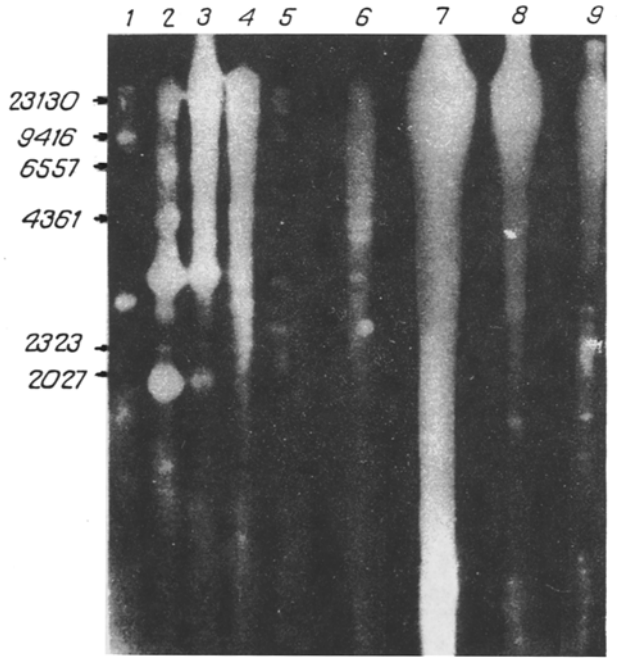

Fig. 3. Blot-hybridization of ^{32}P-DNA from EBV with DNA of cell lines restricted by Hind III. 1, 2, 3, 4, 5) HVGM-producing green monkey cell lines GM-18, GM-19S, GM-19F, GM-20, and GM-91, respectively; 6) HVMA-producing macaque cell line MAL-1; 7) EBV-producing human cell line P3NR1; 8) BHV-producing baboon cell line VPLP-P; 9) BHV-producing cell line $E_{5\text{-}1}$ (a clone of line LUG-4).

from cell line MAL-3. It will be clear from Fig. 2 that with respect to DNA restriction sites lines 20018/MAL-3 and MAL-3 are completely identical (bands 3 and 4). Virus DNA also is found in two-cell lymphoid lines obtained from rabbit tumors, induced by injection of HVMA-containing materials (bands 5 and 6). The results suggest that HVMA is involved in the induction of a malignant lymphoma in rabbits. Differences in the restriction sites of virus DNA in tumor lines from DNA in macaque cell lines also are of definite interest. These results may be evidence of deletions of certain regions of the HVMA DNA in tumor materials leading to loss of the replicative cycle. This is confirmed by the absence of synthesis of virus-specific antigens and the failure to find virus particles in tumor materials, but with preservation of regions of the genome responsible for oncogenic activity of these lines, for these materials preserve the ability to induce tumors in rabbits [7].

The results of blot-hybridization of EBV DNA with DNA of cell lines obtained from green monkeys restricted by Hind III, are shown in Fig. 3. It will be clear from Fig. 3 that HVGM, reproduced in five green monkey cell lines, are characterized by marked intraspecific heterogeneity, and also by differences in their restriction sites from DNA of viruses replicating in macaque, baboon, and human cell lines. However, it is too early yet to link differences in the structure of the genomes of B-lymphotropic herpesviruses of monkeys with their oncogenic activity.

Thus, expression of the corresponding virus genomes of B-lymphotropic herpesviruses of monkeys, differing both in biological characteristics and in homology and DNA restriction sites, has been demonstrated in new biological systems obtained by the present writers (lymphoid cell lines of monkeys and rabbits).

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