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EFFECTIVE METHOD OF INCORPORATING PLASMID DNA INTO EUKARYOTIC CELLS USING LIPOSOMES TO PRODUCE VACCINIA VIRUS RECOMBINANTS

V. A. Slepushkin and M. I. Bukrinskii

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KEY WORDS: recombinant vaccinia virus; liposomes; Sendai virus; AIDS virus.

Vaccinia virus has begun to be widely used recently as a vector for cloning foreign genes [8]. This is because of the high capacity of this vector [cloning of DNA fragments measuring 24 kilobase pairs (kb) has been reported [11]], and the broad spectrum of recipient cells sensitive to the virus. The recombinant virus, carrying genes of microorganisms pathogenic for man and animals, can also be used as a living subunit vaccine [15]. Production of recombinant vaccinia virus expressing HTLV-III-specific proteins is an important stage in the creation of a vaccine against AIDS. A fragment of the env gene of HTLV-III virus was therefore used for cloning.

To obtain recombinant vaccinia viruses, plasmid DNA containing the test gene in an environment of virus-specific sequences has to be incorporated into cells infected with this virus. Usually this can be done by the Ca-phosphate precipitation method [5]. Recently liposomes containing viral or plasmid DNA have begun to be used to transform eukaryotic cells [3, 14]. However, when using this method, investigators have encountered difficulties. Incorporation of DNA into cells with the aid of reverse-phase liposomes, consisting only of lipids, did not give the desired result because of the absence of any mechanism whereby genetic material can pass out of the liposomes into the cell cytoplasm [13]. At the same time, incorporation of DNA into liposomes into which envelope glycoproteins of Sendai virus had been incorporated for the purpose of membrane fusion, proved to be ineffective [4, 14].

An effective method of incorporating plasmid DNA into cells has recently been suggested, based on induction of fusion of the liposome membrane, into which gangliosides has been incorporated, with the plasma membrane of the cell with the aid of UV-inactivated Sendai virus. By means of this method a recombinant vaccinia virus carrying the env gene of HTLV-III virus has been obtained.

EXPERIMENTAL METHOD

Plasmid pGS20 [7] was used as the vector. Recombinant plasmid pGSenvL 1 was obtained by the scheme shown in Fig. 1. The EcoRI/BamHI-fragment of the env gene of HTLV-III virus was obtained after restriction of DNA of plasmid pBH10 [10]. The recipient of the recombinant plasmid was *E. coli* strain HB101 [(F^-, hsdS20(r_B^-, m_B^-), rec A13, lac Y1, rps L20(Str)]. The orientation of the cloned fragment in plasmid pGSenvL 1 was determined after restriction by

*Deceased.

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR. N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Zhdanov.*) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 478-480, October, 1987. Original article submitted October 12, 1986.

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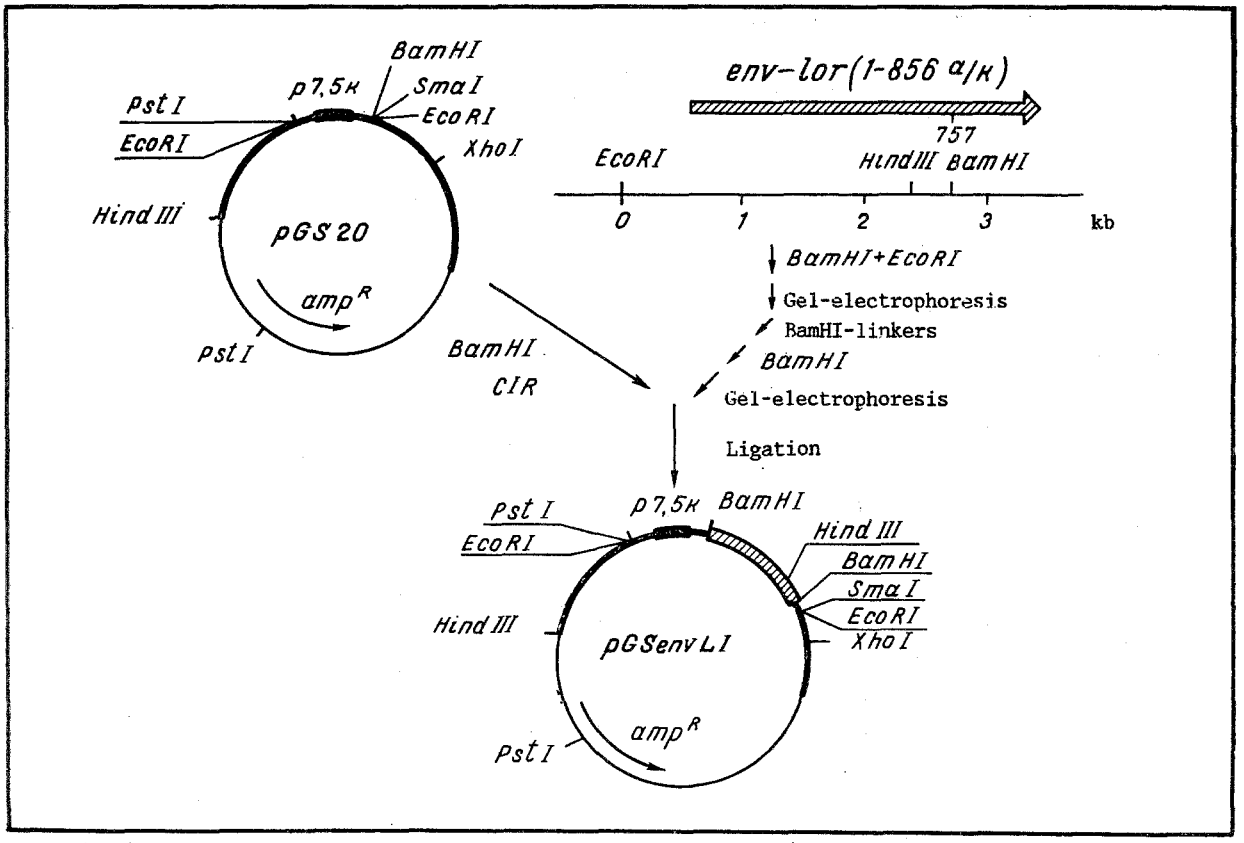


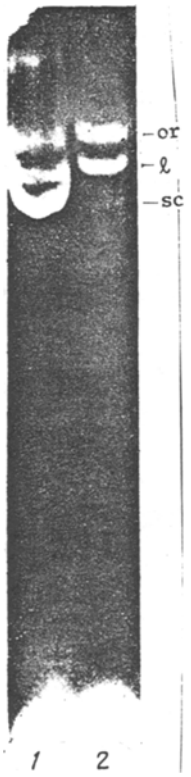
Fig. 1. Scheme of construction of recombinant plasmid pGSenvL 1. Thin line on schemes of plasmids indicates DNA and pBR328 fragments, bold line denotes fragments of vaccinia virus DNA; black shape represents promoter of 7.5k gene of vaccinia virus protein (p7.5k), shaded areas represent cloned fragment of env gene of HTLV-III virus.

HindIII. pGSenvL 1 DNA (50 μg) was isolated from 100 ml of an overnight culture [2] and incorporated into liposomes by the reversed phase method [3]. The liposomes were prepared from egg phosphatidylcholine, cholesterol, and a mixture of disialo-containing gangliosides from bovine brain in the molar ratio of 20:10:1. The gangliosides were isolated from the mixture of glycolipids by ion-exchange chromatography on DEAE-Sephadex A-25 [1]. To incorporate the plasmid into Vero cells (a culture of African green monkey kidney cells) UV-inactivated, concentrated, and purified Sendai virus was added to the DNA-containing liposomes [9]. After incubation at 37°C for 48 h the cells were lysed with 1% sodium dodecylsulfate (SDS) and the DNA was isolated by phenolic extraction. After treatment with restriction endonuclease BamHI the preparation was fractionated in 0.5% agarose gel and transferred to a nitrocellulose filter (Millipore, 0.45 μ) [12]. The DNA immobilized on the filter was hybridized with DNA of the EcoRI/BamHI-fragment of the env gene, labeled by the nick–translation method. Hybridization was carried out at T_m = 27°C (2 × SSC, 65°C) and the filter was washed in 0.1 × SSC (1 × SSC: 0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS twice, for 15 min each time, at 65°C. Fragments hybridized with DNA of the clone were identified by autoradiography, using RM-1 film and EUI-1 intensifying screens, for 16–24 h at –70°C.

EXPERIMENTAL RESULTS

The results of gel-electrophoresis of preparations of DNA of the recombinant plasmid pGSenvL 1, incorporated into liposomes, are shown in Fig. 2. According to our data, 25–30% of plasmid DNA was found in the liposomes. However, during preparation of liposomes many molecules were damaged (single-stranded breaks appeared) and were converted from the supercoils formed into the open ring form.

The effectiveness of penetration of plasmid pGSenvL 1 into Vero cells was judged by the quantity of ^{32}P-DNA associated with them and the appearance of recombinant DNA of vaccinia virus, containing a fragment of the env gene of HTLV-III virus. Approximately 5–10% of labeled plasmid DNA was found to be associated with the cells.



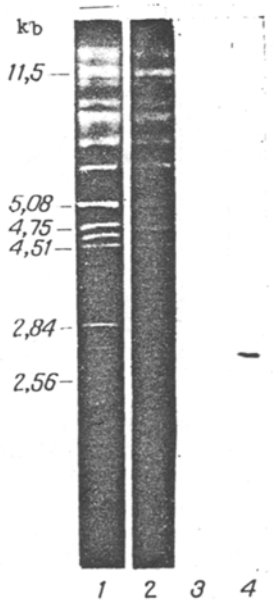


Fig. 2 Fig. 3

Fig. 2. Electrophoretic analysis of pGSenvL 1 DNA incorporated into liposomes. 1) Control preparation of pGSenvL 1 DNA, 2) pGSenvL 1 DNA isolated from liposomes. or) Open ring form of DNA; l) linear form of DNA; sc) supercoil form of DNA.

Fig. 3. Results of block-hybridization of recombinant vaccinia virus, treated with BamHI DNA with ^{32}P-DNA of EcoRI/BamHI-fragment of env gene of HTLV-III virus. 1, 2) DNA in gel stained with ethidium bromide; 3, 4) results of autoradiography. DNA of original vaccinia virus (strain WR) fractionated in lanes 1 and 3. DNA of recombinant virus fractionated in lanes 2 and 4.

The results of experiments on hybridization of ^{32}P-DNA of the EcoRI/BamHI-fragment of the env gene with BamHI-treated DNA from the cells infected with vaccinia virus and transformed by pGSenvL 1 in the composition of the liposomes, are given in Fig. 3. It will be clear from Fig. 3 that a recombinant virus containing a fragment of the env gene of HTLV-III virus, cloned with respect to BamHI, was formed in the cells. This fragment was split up after treatment of the DNA with BamHI (lanes 2 and 4).

The data given above indicate the high efficiency of penetration of DNA in the composition of liposomes into cells and consequent recombination in vivo with vaccinia virus DNA. By this method it was possible to obtain recombinant vaccinia virus carrying a fragment of the env gene of AIDS virus. This result encourages the hope that recombinants synthesizing considerable quantities of HTLV-III-specific protein will be obtained in the near future for use in the diagnosis and prevention of a dangerous disease such as AIDS.

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USE OF MONOSACCHARIDE INHIBITORS TO STUDY THE CYTOSTATIC ACTION OF CYTOTOXIC T LYMPHOCYTES, MACROPHAGES, AND NONADHERENT SPLENOCYTES

B. B. Fuks, I. V. Bogdashin, V. V. Lyakhov, and O. I. Krifuks

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KEY WORDS: peritoneal lymphocytes; macrophages; splenocytes; cytostasis; monosaccharides.

Experiments have shown [9] that during immunization of mice with tumor cells not containing antigens of the H-2 complex, killer-cell activity cannot be found but cytostatic T-cells with the Lyt $1^{+}23^{-}$ phenotype are generated. Without preliminary sensitization the mouse splenocytes can exert a cytostatic action on tumor cells [5]. This effect is linked with the action of the cells themselves and not of soluble factors. These cells are incapable of phagocytosis but they form E-rosettes and have the Fc-receptor. The inhibitory action of monosaccharides and of phosphorylated monosaccharides on the recognition stage has been described in the literature, especially at the lytic stage of action of cytotoxic cells [13, 14]. However, the effect of monosaccharides on the function of cytostatic effectors has not been investigated, and this served as the motivation for the present investigation.

EXPERIMENTAL METHOD

Experiments were carried out BALB/c mice aged 2-4 months. Cytostatic peritoneal lymphocytes (PL) were obtained in BALB/c mice on the 11th day after intraperitoneal injection of EL-4 cells, as described previously [1]. Macrophages were obtained by washing out the peritoneal cavity of the mice with medium containing heparin (10 U/ml) 3 days after intraperitoneal injection of 1.5 ml of a 10% solution of peptone into the animals, followed by adsorption on plastic Petri dishes and removal of the adherent cell population. The fraction of splenocytes not adherent to plastic was used as the effectors. To assess the functional activity of LP the method in [3] was used. The target cells and cold inhibitors were EL-4 and P-815 tumor cells, subcultured in mice.

TABLE 1. Cytostatic Action of Different Effector Cells on P-815 Cells during Incubation for 18 h (M ± m)

Effectors	Ratio effectors/targets	Synthesis inhibition, %	
		DNA	RNA
Macrophages	20:1	51±6	62±7
Spenocytes	20:1	71±6	78±7
PL	10:1	91±4	92±3

Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 480-483, October, 1987. Original article submitted December 22, 1986.

