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Receptor-Mediated Transfer of DNA–Galactosylated Poly-L-lysine Complexes into Mammalian Cells *in vitro* and *in vivo*

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Abstract—With the goal of developing non-viral techniques for exogenous gene delivery into mammalian cells, we have studied receptor-mediated gene transfer using complexes of plasmid DNA and galactosylated poly-L-lysine, poly(L-Lys)Gal. To evaluate the optimal parameters for efficient gene transfer into human hepatoma HepG2 cells by the DNA–poly(L-Lys)Gal complexes, the bacterial reporter genes *lacZ* and *cat* were used. Examination of the reporter gene expression level showed that the efficiency of DNA delivery into the cells depends on the structure of DNA–poly(L-Lys)Gal complexes formed at various ionic strength values. The efficiency of DNA transfer into the cells also depends on DNA/poly(L-Lys)Gal molar ratio in the complexes. Plasmid vector carrying human apolipoprotein A-I (*apoA-I*) gene was injected as its complex with poly(L-Lys)Gal into rat tail vein. Some level of ApoA-I was detected in the serum of the injected rats. Also, the human *apoA-I*-containing plasmid was found to be captured specifically by the rat liver cells and transported into the cell nuclei, where it can persist as an episome-like structure for at least a week. After repeated injections of DNA–poly(L-Lys)Gal complexes, the level of human ApoA-I in rat serum increases, probably, due to accumulation of functional human *apoA-I* gene in the liver cell nuclei. The data seem to be useful for the development of non-viral approaches to gene therapy of cardiovascular diseases.

Key words: gene delivery, mammalian cells, bacterial reporter genes, *lacZ* gene, *cat* gene, human apolipoprotein A-I gene, DNA complex, galactosylated poly-L-lysine, persistence, exogenous DNA, episome-like structure, cell nucleus

The design of delivery vehicles for "therapeutic" gene transfer into somatic tissues and organs is of fundamental importance for progress in gene therapy of different human pathologies. Gene delivery using recombinant viruses (primarily adeno- and retroviruses) is one of the most effective ways. Some significant limitations of this approach are immunogenicity, toxicity (for adenoviruses), and possible side effects (for retroviruses) [1, 2]. There are also non-viral delivery tools [3, 4]; despite their relatively low efficiency, they are characterized by negligible immunogenicity and low toxicity. However, on the whole, a relatively short effect is detected for genes delivered using non-viral agents, for example, by lipofection (liposomes or cationic lipids) [3]. A gene delivery technique using receptor-mediated endocytosis of DNA complexes with molecular conjugates (covalent compounds of DNA carrier polycations and ligands for specific surface receptors) [4] has some advantages over other non-viral tools for selective (directed) gene transfer into cells and, in some cases, for prolonged maintenance of gene constructs delivered into cells [4-8].

Foreign DNA can be delivered into both mammalian liver and cultivated hepatoma cells (HepG2) in a complex with a conjugate consisting of a cationic polypeptide, poly-L-lysine, and a ligand possessing affinity to asialoglycoprotein receptors of hepatocytes [4, 6-8]. Because these are mostly lectin receptors (recognizing a galactose molecule in the carbohydrate component of the desialated protein), the substitution of asialoglycoprotein (ligand) by a galactose molecule in the conjugate does not affect the ability of the receptor to bind the conjugate [4, 7, 8]. It is significant that complexes formed by galactosylated poly-L-lysine with DNA are more compact in comparison with asialoglycoprotein conjugate (the diameters of the DNA–galactosylated poly-L-lysine and DNA–conjugate are 12-15 and 80-100 nm, respectively), thus resulting in a prolonged lifetime of DNA targeted into the liver from several hours to several weeks (possibly months). This fact may be connected with higher probability of targeting of DNA–galactosylated poly-L-lysine compact complexes

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released from endosomes into the cell nuclei and corresponding to prolonged (up to 140 days) expression of DNA targeted into the cells [7-9]. However, it remains unclear what form of foreign DNA (free or integrated with chromosomal DNA) is present in the nuclei.

So, this study was intended to: optimize delivery conditions for foreign gene constructs with the goal of optimizing galactosylated poly-L-lysine targeting into human hepatoma cells carrying asialoglycoprotein receptors; provide foreign plasmid DNA targeting into rat hepatocytes *in vivo* and determine both lifetime and physical condition of exogenous DNA delivered into hepatocyte nuclei. Also, using a gene construct which represents an expression vector of human apolipoprotein A-I (ApoA-I) possessing anti-atherogenic properties [10, 11], the level of ApoA-I in rat blood was supposed to be elevated due to repeated intravenous injections of the gene construct. Consequent elevation of the foreign ApoA-I blood level after repeated injections of the corresponding expression vector offers possibilities for a gene therapy approach to the treatment of atherosclerosis by means of delivery of the *apoA-I* gene in a complex with non-viral carrier *in vivo* [12].

MATERIALS AND METHODS

Human HepG2 hepatoma cells obtained from ATCC/NIH (USA) and murine fibroblast line NIH 3T3 from the Cell Culture Bank of the Institute of Cytology, Russian Academy of Sciences, were used for the experiments *in vitro*.

Male Wistar rats used for the *in vivo* experiments (weight 110-125 g) were obtained from the Rappolovo nursery of the Russian Academy of Medical Sciences (St. Petersburg).

Plasmids pCMVlacZ, pCMVcat, and pCMVapoA-I representing correspondingly the expression vectors for bacterial reporter genes *lacZ*, *cat*, and human *apoA-I* gene under the control of the early cytomegalovirus gene promoter were constructed using gene engineering techniques as described previously [10, 11].

The conjugate of poly-L-lysine with asialated orosomucoid, poly(L-Lys)ASOR, synthesized as described by Perevozchikov et al. [11], was obtained from M. M. Shavlovskii (Institute of Experimental Medicine, Russian Academy of Medical Sciences).

Chemicals used were from Sigma (USA), Serva (Germany), Pharmacia (Sweden), Boehringer Mannheim (Germany), and from Russian manufacturers (analytical or high-purity grade); enzymes for gene engineering were from Fermentas (Lithuania) and Scientific-Industrial Corporation SibEnzyme (Novosibirsk, Russia). Isotopes: [α-^{32}P]deoxy-CTP was from Scientific-Industrial Corporation GIPKh (St. Petersburg, Russia), [^{14}C]chloramphenicol was from Amersham (England).

Synthesis of galactosylated poly-L-lysine. Galactosylated poly-L-lysine, poly(L-Lys)Gal, was synthesized by the method described previously [7]. α-D-Galactopyranosylphenylisothiocyanate (85 μg) was added to 2 mg of poly-L-lysine hydrobromide (mean polymerization degree 100, Sigma catalog No. P 7890) dissolved in 1 ml of phosphate buffer, pH 7.2, followed by the addition of 0.1 ml 1 M Na_2CO_3, pH 9.0. The reaction mixture was incubated with periodic agitation (shaking) in darkness for 16 h at room temperature. Finally, 3-5% of the ε-amino groups of the poly-L-lysine became galactosylated.

Bacterial cell transformation, plasmid production and purification, DNA sample labeling. Transformation of bacterial cells *E. coli* K12 (strains HB101, XL1-blue) with DNA was carried out by electroporation; plasmid DNA production, purification, and labeling of the isolated DNA samples were carried out in accordance with the methods described by Sambrook et al. [13].

Formation of DNA–galactosylated poly-L-lysine complexes. The DNA–poly(L-Lys)Gal complexes formed were titrated as follows. Increasing quantities of poly(L-Lys)Gal dissolved in 0.7 M NaCl were dropped under thorough agitation into aliquots of plasmid DNA (1 μg of DNA purified by ultracentrifugation in a CsCl gradient and dissolved in 10 μl of 0.7 M NaCl). Equal portions of each sample were placed into the starting wells on a 1% agarose gel, and electrophoresis was carried out. Retardation of DNA–poly(L-Lys)Gal complexes was tested after the electrophoresis according to the routine method [14].

The DNA–poly(L-Lys)Gal complex for transfection of cells cultivated *in vitro* or for intravenous injection into animals was prepared in accordance with the following method. DNA and poly(L-Lys)Gal were dissolved in equal volumes of 0.4 M NaCl. The solution of galactosylated poly(L-Lys) was dropped into the DNA solution with thorough agitation after addition of each drop to prevent the formation of a pellet. Then, 5 M NaCl was added to the solution of the complex formed to the final concentration of 1.1 M, and the solution obtained was used for cell transfection or intravenous injections into animals.

Transfection of cultivated cells and analysis of expression of bacterial reporter genes in transformed cells. HepG2 and NIH 3T3 cells were cultivated to 70% confluence in Petri dishes (60-mm diameter) in DMEM medium with 10% fetal calf serum (Gibco, USA), at 37°C in a CO_2 incubator. Cells were transfected with the DNA–poly(L-Lys)Gal preparations in accordance with our own protocol based on the method of Erbacher et al. [15]. For this purpose, the cells were incubated for 48 h in 2 ml of DMEM medium containing 10 μg of DNA in a complex with poly(L-Lys)Gal, 10% of calf fetal serum inactivated by heating (Gemini Bioproducts, USA), 10 mM $CaCl_2$, and 100 μM of chloroquine (Sigma).

The efficiency of transfection was evaluated by bacterial enzyme activity determination of the products of the *lacZ* and *cat* genes. β-Galactosidase activity in cells transfected with the pCMVlacZ was determined using chromogenic substrates, X-Gal, or *o*-nitrophenyl-β-D-galactopyranoside (Sigma) [16]. The Cat assay was carried out according to the method proposed by Gorman [17].

Injection of the DNA–poly(L-Lys)Gal complexes into rats. DNA complexes (pCMVapoA-I) with poly(L-Lys)Gal prepared as described above were injected into the rat tail vein (25 μg of DNA per animal). The rats were sacrificed after the experiment in accordance with the conventional rules (using anesthetics).

DNA isolation from animal tissues and foreign nucleotide sequence analysis in bulk rat DNA. To analyze foreign nucleotide sequences, chromosomal DNA was isolated from cells of various rat tissues using the standard method [13]. Low-molecular-weight nuclear DNA was isolated from hepatic cells according to the method of Hirt as described by Brown and Scott [18].

Targeting of the construct containing human *apoA-I* gene into rats was confirmed by DNA analysis by means of PCR using primers to a human ApoA-I cDNA (550 b.p.) selected using the software PRIMER (authors V. Prutkovsky and O. Sokur, Influenza Institute, Ministry of Health of the Russian Federation). Forward primer: 5′-CCTGGGATCGAGTGAAGGAC-3′, reverse primer: 5′-CGTGCTCAGATGCTCGGTGG-3′.

Analysis of the human *apoA-I* gene expression product. Human ApoA-I in rat blood sera was measured by immunoenzyme assay (ELISA) with rabbit polyclonal antibodies against human ApoA-I and secondary goat antibodies against rabbit IgG conjugated with horseradish peroxidase. Diaminobenzidine was used as a chromogenic substrate.

RESULTS AND DISCUSSION

Transfection of cultivated hepatoma cells with DNA–poly(L-Lys)Gal complexes. DNA–poly(L-Lys)Gal complex formation was determined by retardation of DNA migration in 1% agarose gel (Fig. 1). DNA–poly(L-Lys)Gal complexes are formed due to electrostatic interaction of the polycation, poly(L-Lys), with the polyanion, DNA; thus, complex formation results in a decrease in negative charge and hence a decrease in DNA migration rate (retardation in gel). As judged from the literature, complete DNA retardation at the starting wells in the gel of DNA–poly(L-Lys) complexes, including DNA–glycosylated poly(L-Lys) complexes, corresponds to DNA charge neutralization at nucleotide/lysine ε-amino group molar ratio 1 : 1 [5, 7, 8, 14]. Complex charge becomes obviously positive as the conjugate content in the complex further increases. In our experiments on plasmid DNA (pCMVapoA-I) titration by increasing quantities of galactosylated poly-L-lysine, complete negative charge neutralization evaluated by mobility in an electric field was observed at DNA/poly(L-Lys)Gal molar ratio of 1 : 156, which is close to calculated value.

In most studies on cell-target transfection with DNA conjugates including various kinds of glycosylated poly-L-lysine, the complexes used were formed after neutralization of the negative charge of DNA [4, 7, 8, 14]. Hence, it was interesting to elucidate whether further increase in the molar portion of galactosylated poly-L-lysine in the complex would influence the transfection efficiency, when the portion is over the value necessary for neutralization of DNA negative charge, thus providing complexes with positive charge. The efficiency of transfection of HepG2 cells with pCMVcat–poly(L-Lys)Gal complexes, as evaluated by Cat-test results (Fig. 2), was substantially increased at 10-fold increased molar portion of galactosylated poly-L-Lys in comparison with the quantity which was necessary to neutralize the DNA charge (molar ratio between DNA and galactosylated poly-L-Lys was 1 : 1120 and 1 : 112, correspondingly). Similar results were obtained when HepG2 cells were transfected with pCMVlacZ–poly(L-Lys)Gal complex at various molar ratios between DNA (pCMVlacZ) and galactosylated poly(L-Lys) (Fig. 3). In these experiments, increasing the molar excess of poly(L-Lys)Gal over DNA led to

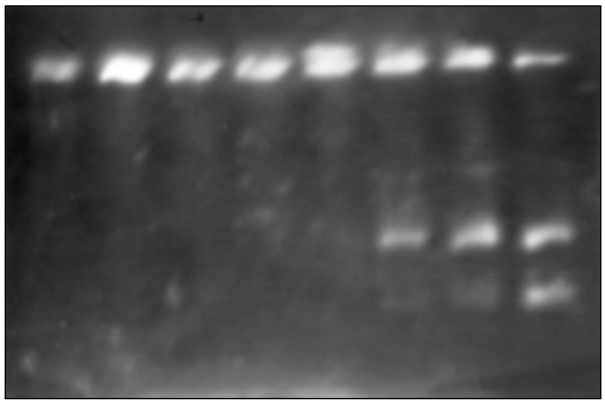

Fig. 1. DNA–poly(L-Lys)Gal complex retardation in a gel during electrophoresis. Retardation of newly formed complexes between DNA (gene construct pCMVapoA-I) and poly(L-Lys)Gal was tested in 1% agarose gel during gel-retardation experiments. Complexes were formed in 0.7 M NaCl as described in "Materials and Methods". Lanes *1-8* represent DNA–poly(L-Lys)Gal complex positions at various molar ratios between DNA and galactosylated poly-L-lysine: *1*) 1 : 312; *2*) 1 : 273; *3*) 1 : 234; *4*) 1 : 195; *5*) 1 : 156 (corresponds to neutralization of charge in the complex); *6*) 1 : 117; *7*) 1 : 78; *8*) 1 : 39.

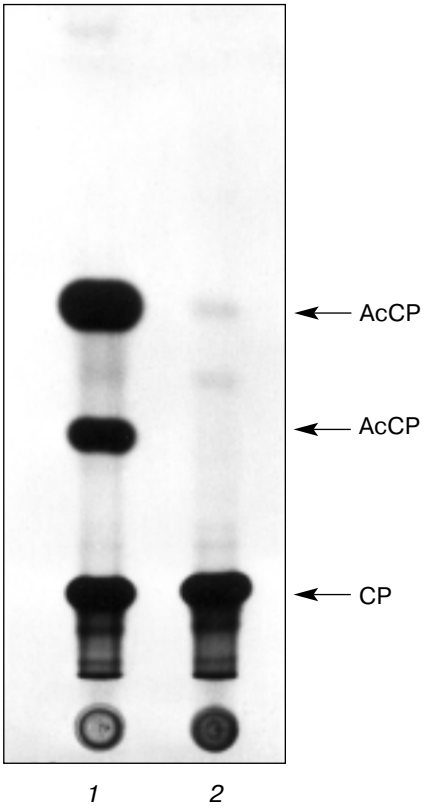

Fig. 2. Results of the Cat assay for gene construct pCMVcat delivered into HepG2 cells as a complex with poly(L-Lys)Gal. Complexes were formed in 0.4 M NaCl. The Cat-test was performed as described in "Materials and Methods". The molar ratios between DNA and poly(L-Lys)Gal in the complexes used for transfection were 1 : 1120 (*1*) and 1 : 112 (*2*) (corresponding to neutralization of charge in the complex). CP, [^{14}C]chloramphenicol; AcCP, acetylated derivatives of chloramphenicol.

proportional increase in β-galactosidase activity in the cell lysates. A beneficial effect of the increase in poly(L-Lys)Gal molar portion in the complex with DNA resulting in an increase in the positive charge of the complex may be favorable for better contact of the latter with the negatively charged cell membrane [5, 15]. We failed, however, to obtain any significant efficiency of transfection of NIH 3T3 murine fibroblasts, which are devoid of asialoglycoprotein receptors, with positively charged pCMVlacZ–poly(L-Lys)Gal complexes (data not shown). Hence, a more probable explanation for the enhancement of HepG2 cell transfection efficiency on increasing the molar portion of galactosylated poly-L-lysine in the complex with DNA may be the corresponding increase in the portion of the ligand binding to the specific receptor. This is in consistence with the data of Erbacher et al., who observed elevation of expression level of the reporter gene targeted into HepG2 cells as a complex with lactosylated poly-L-lysine with increasing extent of lactosylation of poly-L-lysine from 20 to 40% or with increase in the molar ratio between lactosylated poly-L-lysine and DNA in the complex [15]. It should be noted that the transfection efficiency for foreign DNA with glycosylated poly-L-lysine depends also on the size of the DNA transfected [8, 15].

The key role of asialoglycoprotein receptors in DNA–poly(L-Lys)Gal complex internalization by hepatoma cells is confirmed by the results of experiments on concurrent exclusion of poly(L-Lys)Gal-labeled DNA complexes from binding sites of HepG2 cells by increasing quantities of poly(L-Lys)Gal or poly(L-Lys)ASOR complexes with unlabeled DNA (Fig. 4). Asialoorosomucoid (ASOR) is a desialated glycoprotein that selectively interacts with asialoglycoprotein receptors and is used in molecular conjugate design [4, 14].

Thus, the results presented here confirm the specificity of interaction of DNA–poly(L-Lys)Gal complexes with asialoglycoprotein receptors on HepG2 cells.

When studying the physical and chemical properties of DNA–poly(L-Lys)Gal complexes, Perales and

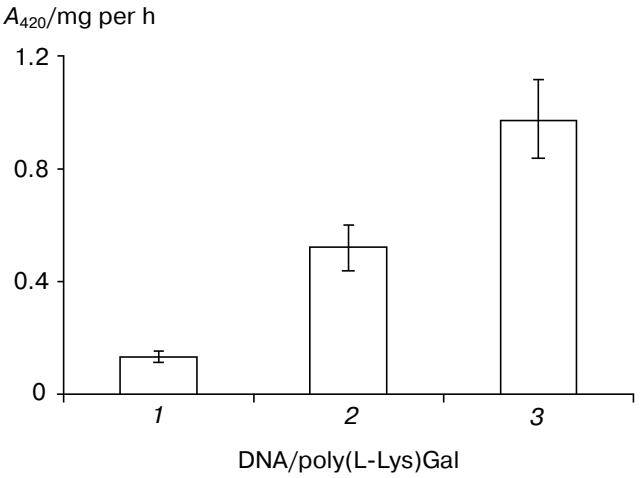

Fig. 3. Effectiveness of HepG2 cell transfection with pCMVlacZ–poly(L-Lys)Gal complexes depending on molar ratio between DNA and galactosylated poly-L-lysine in the complex. The effectiveness of DNA transfer was evaluated by the level of activity of the expression product of gene *lacZ* encoding bacterial β-galactosidase in cell lysates. *o*-Nitrophenyl-β-D-galactopyranoside was used as a chromogenic substrate. Ordinate, relative units of enzymatic activity (calculated from the optical absorption increase at 420 nm during the course of the reaction catalyzed by β-galactosidase per mg protein per h). DNA (10 μg of pCMVlacZ) was used for transfection of HepG2 cells growing on Petri dishes (60-mm diameter). Molar ratios DNA/poly(L-Lys)Gal in the complexes used for cell transfection were 1 : 100 (*1*) (corresponds to neutralization of the charge in the complex), 1 : 1000 (*2*), and 1 : 3000 (*3*).

coworkers established that their soluble (condensed) state corresponds to a monomolecular structure of DNA electrostatically bound to define quantities of cationic polypeptide molecules [8]. Such complexes optimally formed at 1.1 M NaCl concentration are most effective for transfection of test cultures. We also determined the efficiency of HepG2 cell transfection by the pCMVlacZ–poly(L-Lys)Gal complexes (at 1 : 100 molar ratio between DNA and poly(L-Lys)Gal in the complex) formed at various NaCl concentrations. Transfection efficiency evaluated by β-galactosidase activity in cell lysates was maximal when the complex was formed in NaCl concentration of 1.1 M, and then decreased drastically, in agreement with data of the authors mentioned above (results not shown).

Using complexes formed at 1.1 M NaCl concentration and high poly(L-Lys)Gal/DNA molar ratios for cell transfection, we found that the HepG2 cell transfection efficiency calculated by β-galactosidase activity (with X-Gal as the substrate) is no less than 10-15%, a value comparable with the transfection efficiency with the same cells by calcium phosphate precipitation.

Delivery of the complex of human apolipoprotein A-I gene with galactosylated poly-L-lysine into rat liver. The human *apoA-I* gene for ApoA-I protein that, when secreted into the blood, is responsible for association of high density lipoproteins and an anti-atherogenic effect [10, 11] was used as a reporter for monitoring the efficiency of foreign gene construct targeting into mammal liver.

The lifetime of foreign DNA delivered by poly(L-Lys)ASOR conjugate into hepatic mammalian cells is known to be very limited (24-48 h), which is probably connected with the hydrolysis of DNA–poly(L-Lys)ASOR complexes released from endosomal vesicles by cytoplasmic nucleases [4, 14]. Unlike poly(L-Lys)ASOR, galactosylated poly-L-lysine forms with DNA complexes of substantially smaller size, the maximal diameter of the spherical structures being 12-15 nm [7, 8]. The small size of conjugate complexes with DNA is favorable for long-term DNA storage due to their transfer into the cell nuclei and corresponding prolonged (up to 140 days) expression of DNA delivered into the cells [7]. However, it remained unclear what form of foreign DNA (free or integrated with chromosomal DNA) was present in the nuclei. In connection with this, experiments were carried out to elucidate the state of the foreign DNA targeted into rat hepatocytes.

pCMVapoA-I–poly(L-Lys)Gal complexes designed for injection into rats were formed at DNA/galactosylated poly-L-lysine molar ratio 1 : 156 as described in "Materials and Methods".

Three groups of rats (five animals in each group) were injected into the tail vein with pCMVapoA-I–poly(L-Lys)Gal complexes (25 μg of DNA per rat per injection). Rats from the first group were injected once at the beginning of the experiment (on the first day); rats from the second group were injected at daily intervals with a total of four injections. Rats from the third group were injected with the DNA–poly(L-Lys)Gal complexes on the sixth day of the experiment. Intact rats were used as controls. All the animals were sacrificed on the eighth day of the experiment.

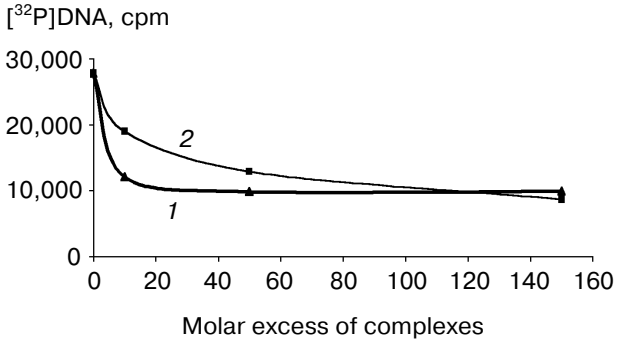

Fig. 4. Specificity of DNA–poly(L-Lys)Gal complex binding to asialoglycoprotein receptors of HepG2 cells. HepG2 cells were incubated on Petri dishes (30-mm diameter) in 1 ml of DMEM medium for 2 h at 4°C with complexes formed between [32P]DNA (pCMVlacZ) and poly(L-Lys)Gal in the presence of increasing amounts of non-labeled DNA–poly(L-Lys)Gal complexes (*1*) or DNA–poly(L-Lys)ASOR (*2*). The medium was removed from the dishes after 2 h. The cell monolayer was washed once with cooled DMEM medium followed by washing 3 times with DMEM with the addition of 0.5% BSA and once with PBS, pH 7.4. NaOH (1 ml, 0.1 M) was added to each dish, and radioactivity was measured in aliquots of protein extracts after 24 h.

Addressed targeting of the complexes into the rat liver was confirmed by DNA analysis of various rat tissues by the PCR method. The nucleotide sequence of the human *apoA-I* gene was identified in bulk rat DNA isolated from hepatic cells (Fig. 5c), but not from small intestine, lungs, or heart (PCR data not presented).

The results on targeting of the plasmid expression vector (pCMVapoA-I) of human *apoA-I* gene by rat tail vein injection of DNA in a complex with poly(L-Lys)Gal are shown in the Fig. 5. The data on "saving" of the plasmid pCMVapoA-I from low-molecular-weight nuclear DNA of hepatic cells isolated by the method of Hirt (see "Materials and Methods") are of special interest. Competent bacterial cells of *E. coli* XL1-blue were transformed with low-molecular-weight nuclear DNA of the rats after intravenous injection with the complexes. The efficiency of bacterial cell transformation by the electroporation method used in the work presented was $5 \cdot 10^8$ clones per μg of DNA. The number of clones that grew up after the transformation of *E. coli* cells with low-molecular-weight nuclear DNA from rats injected several times with the complexes exceeded the number of clones that grew up after cell transformation with low-molecular-weight nuclear DNA from rats injected only

pCMVapoA-I–poly(L-Lys)Gal complex

a

Injection into the rat tail vein

Number of rat	1-5 I group	6-10 II group	11-15 III group	16, 17
Number of injections	1 (1st day of experiment)	4 (1st, 2nd, 4th, 6th days of experiment)	1 (6th day of experiment)	control
Human ApoA-I in rat serum, ng/ml	29 ± 7.5	90 ± 9.7	38 ± 7.1	0

Isolation of low-molecular-weight DNA from cellular nuclei

b

Electroporation of bacterial cells XL1-blue, analysis of plasmids by the PCR method

c

PCR analysis of human *apoA-I*

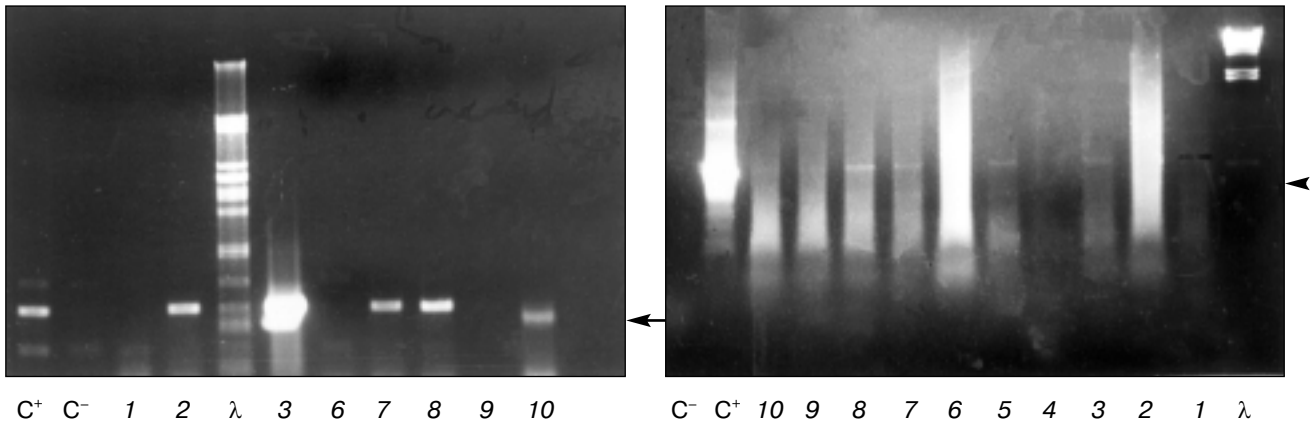

Fig. 5. Targeting of the gene construct expressing human *apoA-I* gene into rat liver. a) Results on human ApoA-I determination by enzyme immunoassay in rat serum after injection of pCMVapoA-I–poly(L-Lys)Gal complexes on the indicated day of the experiment. ApoA-I concentration means and standard deviations are given in the table. The rats were sacrificed on the eighth day of the experiment, nuclei were isolated from hepatic cells, and low-molecular-weight nuclear DNA was separated as described in "Materials and Methods". b) Results of PCR on human *apoA-I* gene determination in clones that grew up after bacterial cell transformation by low-molecular-weight nuclear DNA preparations isolated from rat liver. C^+, plasmid DNA (pCMVapoA-I) containing cDNA of the human *apoA-I* gene (positive control); *1-10*) plasmid samples corresponding to the rat numbers presented in the upper table (a); λ, phage λ DNA digested by *Pst*I; C^-, negative control. c) Results of PCR on human *apoA-I* gene determination in low-molecular-weight nuclear DNA of hepatic cells (fraction marked by the arrow on the right side of the electrophoregram); *1-10*) samples of low-molecular-weight nuclear DNA corresponding to the rat numbers presented in the upper table (a); λ, phage λ DNA digested by *Hind*III; C^+, pCMVapoA-I (positive control); C^-, negative control.

once (nevertheless, we failed to observe any statistically significant differences). Plasmid DNA was isolated from bacterial cell clones numbered corresponding to the numbers of the rats injected with the complexes and analyzed by the PCR method (Fig. 5b). As evident from the results presented here, the nucleotide sequence specific for human *apoA-I* is present in both low-molecular-weight nuclear DNA from injected rat liver (Fig. 5c) and in plasmids "saved" from hepatic cell nuclei of injected rats by means of bacterial cell transformation with low-molecular-weight DNA (Fig. 5b). Moreover, the data indicates prolonged (during eight days) maintenance of pCMVapoA-I in hepatic cell nuclei in a form of autonomic DNA. The results of PCR analysis also indicate substantial individual differences in quantities of foreign DNA targeted into the hepatic cells as a result of intravenous injections (see also the data obtained by Perevozchikov et al. [10]). The reason for these variations is still not clear.

Expression of the *apoA-I* gene in hepatic cells was judged by the content of human ApoA-I in blood serum of animals from the three groups, since the protein expression product of gene *apoA-I* is secreted into blood after its synthesis in cells [10, 11]. Human ApoA-I was identified in the sera of rats from all three groups (Fig. 5a). Human ApoA-I concentrations in the blood serum of animals from the first group differed non-significantly from the corresponding values in animals from the third group, thus indicating (when taking into account the short ApoA-I half life in blood plasma) prolonged expression of the transferred gene construct stored in the hepatocyte nuclei. Repeated injections of the complex into the animals (second group) result in elevation of the human ApoA-I level in rat blood serum in comparison with the human ApoA-I level in blood sera of animals from the first and the third groups; this may be a consequence of increased amount of expressed ApoA-I mRNA in proportion to the *apoA-I* gene dose increasing in rat liver resulting from repeated injections. Taking into account the fact that delivery of the *apoA-I* gene into an animal with sclerotic damage of blood vessels results in regression of this damage [12, 19], the development of non-viral techniques for delivery of the gene encoding anti-atherogenic ApoA-I into mammalian cells *in vivo* may serve as a basis for sparing approaches to cardiovascular disease treatment by means of gene therapy.

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