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REVIEWS

Baculovirus vectors in experimental gene- and vaccine therapy

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The article provides a brief overview of the literature on target design, exploration properties and effectiveness of the application of recombinant baculoviruses in model systems in vivo. The results of experiments with wild and recombinant baculoviruses are analysed in regard to the priority areas of biomedicine such as tissue regeneration, gene therapy of cancer, development of vaccines against infe tious diseases and malignancies.

Keywords: baculovirus, gene- and immunovector, mammals, system in vivo.

Intensive development of investigations and progress, achieved in the sphere of experimental gene therapy, are greatly conditioned by the design of efficient ways and means of delivery of genetic material into cultivated mammalian cells *in vitro*. However, the solution of a key task of this biotechnology – efficient delivery and expression of target gene-therapeutic constructions into an organism – requires the development of new vector systems with specific properties. It is related to the fact that the process of transport of genetically engineered constructions – usually high molecular complexes such as recombinant viruses or plasmid DNA – is very complicated in an organism and mediated by both the necessity of overcoming a number of physiological and structural barriers and certain protection of the vectors from the the immune system action.

Therefore, one of the most important tasks of developing this direction in biotechnology is the creation of the target low-toxicity transport vectors, *i.e.* the main instrument of gene therapy. Here great relevance is attributed to the necessity of delivery of the required number of therapeutic genes or antigen proteins into the target cells, organs, and tissues with low-toxicity of the vector and optimal level of expression. The primary hindrance for a wide and efficient application of the most used vectors in gene therapy is the induction of natural immune response to systemic introduction of these carriers. The solution of this problem is the creation of productive ways of overcoming vector-mediated prolonged non-specific

Features of vectors used for target deliver of genes

Vector	Cloning volume, thousand b.p.	Tropism	Integration into genome	Duration of transgene expression	Induction of immune response	Note
Retrovirus	8	Dividing cells	Integrates	Prolonged	Weak	Risk of insertion mutagenesis, low titres
Lentivirus (Lentivirus)	8	Wide tropism	Integrates	Prolonged	Weak	Risk of insertion mutagenesis, high efficiency of transduction
Adenovirus (Ad)	Up to 36	Wide tropism	Does not integrate	Short	High	Strong immunogenicity, high titres
Adeno-associated virus (AAV)	Less than 5	Wide tropism	Integrates	Prolonged	Weak	Demands adenovirus as a helping agent, low titres
Herpes virus (HSV)	Up to 30	Wide tropism	Does not integrate	Prolonged	High	High titres, highly infectious, complicated regulation
Plasmid DNA	Over 20	Wide tropism	Depending on DNA construction	Up to several months	Weak	Produces easily, low efficiency of transduction
Nuclear polyhedrosis virus (NPV) of insects	Over 30	Wide tropism	Does not integrate	From 3 to 60 days	Weak	High titres, cheap scaling

immune response, consisting in the activation of synthesis of a number of pro-inflammatory cytokines, maturing of antigen-presenting cells and damage of tissues which decreases the period of transgene expression and results in undesirable side effects.

At present many laboratories in the world perform intensive studies aimed at the application of viral and non-viral recombinant vectors for purposes of gene therapy and cell engineering. Viral vectors provide a more effective and sufficiently targeted transfer of functional genes compared to non-viral systems. It means the affinity of viral vectors to certain tissues (let us say, the vectors on the basis of herpes simplex virus are tropic to nerve tissue) and the presence of a nuclear signal, providing a high level of expression of introduced genes in the cell nucleus. Numerous studies

support the most active application of retrovirus, lentivirus, adenovirus, adeno-associated vectors, demonstrating good clinical perspective. The detailed structure of abovementioned base virus vectors and the sphere of their application in tissue engineering and anti-inflammatory therapy are described in the reviews [1, 2]. However, they have some drawbacks, limiting their further design (Table).

As seen from Table, the vectors, designed on the basis of human viruses, have a number of considerable shortcomings, including those related to the presence of pre-existing antibodies with rather high toxicity [3]. This situation motivated the search for and creation of new virus vectors that are non-pathogenic for humans and animals. At present the most promising baculovirus is considered to be *Autographa californica* and some related baculoviruses. They interact efficiently with different types of mammalian cells, using penetration mechanisms, similar to those for penetration into insect cells [4, 5]. These baculoviruses are available for gene engineering and chemical modification of a viral particle surface which gives a real possibility of presenting target peptides and proteins on it.

Nuclear polyhedrosis virus (NPV) of Lepidoptera is among new and quite intensively elaborated virus vectors for mammalian cells and tissues. NPV of *Autographa californica* has been studied in the most detailed way and widely used for genetic modifications and in expression systems. The virus contains double-stranded genome DNA, the size of nucleocapsid varies from 40 to 50 nm in diameter and from 200 to 400 nm in its length. Due to rather a large number of insects that can be infected, this baculovirus is efficiently applied as a bioinsecticide and in the construction of highly-efficient genetic expression systems along with other baculoviruses [6].

The baculovirus vector system is often used to produce a considerable number of eu- and prokaryotic proteins in the culture of insect cells. The development of technology of modifying the baculovirus surface allows presenting foreign peptides and even complexes of proteins on the envelope or capsid of the virus [7]. High cloning volume of the genome of baculovirus, the possibility of obtaining recombinant virus in high titres, the capability of sufficiently high level of penetration into dividing and non-dividing mammalian cells with no replication in them, as well as low toxicity make this vector quite a promising means for the purposes of cell engineering and gene therapy [4].

Recombinant baculoviruses in cell therapy and tissue engineering. Gene engineering of cells is an important component of tissue engineering, performed outside of the organism. Genetically modified cells are introduced into the organism via different ways and directly implanted into the required place. In this respect special attention is paid to stem cells (SC) of various origin, and first of all, to embryo stem cells (ESC) and mesenchymal stem cells (MSC). MSC of bone marrow, capable of differentiating in various directions and migrating to lesions in case of corresponding signals, attract increased attention of scientists, working in the field of the regenerative medicine and anti-tumour therapy.

The aim of regenerative medicine is restoration of functions of damaged organs and tissues by transplantation of either cells or “bio-artificial” organs and tissues constructed outside the organism (*ex vivo*) on synthetic or natural matrices. A new, intensively developing sphere of biotechnology, which uses the achievements of gene engineering and is an important constituent of regenerative medicine, namely tissue engineering, – is is aimed at overcoming the deficiencies of traditional approaches. This is perfectly described in the work [8] : “All procedures, restoring lost tissues of a patient, require a specific type of substitutional structures in the area of defect or damage. Traditionally these appliances were completely artificial (joints), transformed non-living tissue (heart valves) or tissue from another place, taken from this patient or from other patients (transplantation). At present the clinicians have a new available alternative – tissue engineering: replacement of living tissues with living tissues which were elaborated and constructed in accordance with individual needs of each patient”

Cell gene therapy is quite a promising strategy for regeneration of damaged musculoskeletal tissues. Its experimental application demonstrated high efficiency in studies on model animals compared to the usual approaches of osteoplasty. A good example is the application of modified stem cells with the genes of bone morphogenic proteins. Among the most known and used factors of induction of bone tissue regeneration there are bone morphogenic proteins (BMP), some representatives of the family of growth factors: transforming growth factor TGF-в, insulin-like growth factor IGF, growth factor of vascular endothelium VEGF.

Recombinant human osteoinductive bone morphogenic protein 2 (BMP2) is widely used in the clinical practice for restoration of damaged bone tissue. Along with the positive results of application of recombinant protein, a significant drawback of this therapy, rather short lifetime of this preparation, is revealed. The introduction of milligram amounts of the inductor is required which is toxic for the organism. The advantage of gene therapy is a possibility of delivering physiological amounts of therapeutic protein directly to the place of damage using different vector systems. This technology allows applying much lower doses of this factor to achieve the effect of bone restoration, equal to that, achieved by systemic introduction of recombinant factor.

MSC are easily transduced by virus vectors, which allows using them for the delivery of a therapeutic gene. It was established that proteins BMP2, BMP4, and BMP7 initiate and support osteogenesis [9]. For instance, BMP was shown to induce fast restoration of large-size cranial defects in primates and rodents [10]. MSC of humans, transduced by recombinant adenovirus, expressing BMP2, excelerate the regeneration of femoral bones of rats [11].

Low-toxic baculovirus, non-pathogenic regarding mammals, is a promising candidate for the work in regenerative medicine. *In vitro* experiments revealed that baculovirus *A. californica* is rather efficient in transduction of articular chondrocytes of rats and rabbits in cell cultures [12, 13]. While the recombinant baculovirus with the marker fluorescent protein EGFP was used in the first work, the recombinant baculoviruses with the genes of growth factors TGF-1, IGF-1, and BMP-2 were used in the second one. The transduced cells were cultivated using two- or three-dimensional matrix. The experiments revealed that both the level of expression of growth factors by recombinant baculovirus and the cell response to it depend on the passage of cells and are kept on the level, sufficient to reveal therapeutic effect. At the same time it was noted that the expression of growth factor BMP2 by the baculovirus enhances the production of intercellular matrix.

Further studies of the influence of growth factors on re-differentiation of rabbit chondrocytes in the cell culture demonstrated the enhancement of this process in case of combined injection of two recombinant baculoviruses with the genes of growth factors BMP2 and TGF-1 [14]. However, super transduction, *i.e.* reintroduction of recombinant baculovirus, reveals short-term dose-dependent activation of synthesis of interferons б and в which suppresses transgene expression to some degree [15]. Nevertheless, even in these conditions the morphogenetic protein BMP2 is synthesized at a sufficiently high level, thus providing the production of intercellular matrix and increase in differentiation of chondrocytes.

At the following stage of the development of regenerative tissue engineering the special modified bioreactors were used to obtain comprehensive implants of cartilaginous tissue as a result of transduction of chondrocytes by recombinant baculovirus. Due to this optimization of technological approach it was possible to create efficient implants, restoring the hyaline structure of cartilaginous tissue in experimental animals [16].

There are parallel studies connected with the development of potential of MSC, genetically modified by recombinant baculovirus, for the purposes of tissue engineering. The capability of MSC to differentiate into osteoblasts, chondrocytes, adipocytes, myocytes and nervous cells under corresponding conditions makes it quite a promising object for using in

regenerative medicine and tissue engineering after genetic modification [17].

High efficiency of MSC transduction by recombinant baculovirus (up to 95%) and invariability of their differential potential after this procedure [18] permitted to demonstrate the induction of MSC differentiation into osteoblasts with subsequent regeneration of a bone defect after their transduction by baculovirus with the gene of protein BMP2 and transplantation into mice [19].

This work was proceeded with the purpose of determining immunostimulatory features of MSC, transduced *ex vivo* by non-recombinant baculovirus and transplanted into animals. It was important to estimate the availability of stem cells, transduced by baculovirus, in the cell gene therapy. It was established that besides local, weak response, transplantation of SC, transduced by baculovirus, does not induce monocytes and $CD8^+$T cells. Therefore, an important result of this research is the conclusion that baculovirus induces a moderate and short-term response in MSC. It allows foreseeing their efficient and safe modification for gene therapy [20].

Remarkable results were obtained for reparation of rather a large (10 mm) bone defect in rabbits. Co-transplantation of bone marrow MSC, transduced by two recombinant baculoviruses with the *BMP2* and *VEGF* genes, resulted in fast restoration of the defect in all the experimental animals and active angiogenesis in the damaged area [21].

A hybrid baculovirus vector was developed by the authors on the basis of FLP/Frt-mediated recombination with the formation of episome for prolongation of the expression of transgene by recombinant baculovirus. FLP/Frt is a system for artificial homologous recombination from the *Saccharomyces cerevisiae* cells. It is based on the application of genetically engineered constructions, containing so called "hot spots", or sites of mitotic recombination – Frt, and the gene of FLP-recombinase, for which these sites are a place of revealing their activity. The result is the formation of the episome, where the sequence oriP/EBNA1 of Epstein-Barr virus is used to support its replication and segregation between daughter cells. The authors constructed two recombinant baculoviruses – one with *flp* gene, and the second– with the construction, containing oriP/EBNA1 and flanked by Frt-sequences. After transduction with these recombinant baculoviruses a self-replicating ring episome is formed as a result of the excision/recombination process. The transduction of human MSC by this hydrid vector with *bmp2* gene allows considerable prolongation of the time of transgene expression (up to 60 days) and thus enhanced efficiency of differentiation of bone tissue cells [22].

From the standpoint of applying MSC, transduced by a hybrid baculovirus vector, in the clinical practice, experimental evaluation of their biosafety is quite important. The data on insignificant inhibition of the MSC cells by this vector, were confirmed. It is most likely determined by the abovementioned changes in the expression of a number of cellular genes and the activation of cellular immune response which nevertheless does not affect the cell viability and differentiation. We also confirmed the data on the absence of both transgene integration into the genome of MSC and change in their karyotype. We revealed no activation of either protooncogenes or genes of tumour suppressors. MSC, transduced by hybrid baculovirus, do not induce the tumour formation in athymic rats. Therefore, the abovestated data testify to the safety of human MSC for gene therapy and availability of MSC, genetically modified by hybrid recombinant baculovirus, in the therapy, requiring prolonged expression of a target gene [23]. The engineering of mammalian cells on the basis of virus vectors, including baculovirus, is one of the most actively developing gene therapeutic strategies. The application of genetically modified cells, stem cells in the first place, allows active and targeted influence on the processes of tissue regeneration. The usage of recombinant baculoviruses together with target genes of growth factors and differentiation inductors is really promising and has considerable advantages compared to other vectors due to their low toxicity and significant potential of enhancing the expression of target proteins

via gene engineering and chemical modification of the surface of viral particles.

Experimental vaccine therapy. One of the first references on applying the recombinant baculovirus as an immune vector is dated 1992 [24]. As a vaccine, the authors used both recombinant baculoviruses, expressing genes *gag* and *env* of leukemia of cats (FeLv), and insect cells (Sf9 line), infected by them (notably, it is one of the first mentions of application of insect cells, infected by recombinant baculovirus, as vaccine). According to the authors, the experiments were successful. However, there was no control – the response to the introduction of non-recombinant, wild virus in the scheme of experiment. It appeared to be a considerable drawback of the work, as later it was shown that the interaction of non-recombinant baculovirus *A. californica* with murine and human cells in the culture induces the synthesis of interferons β and γ, and therefore promotes antiviral defense *in vivo* for infection of mice with encephalomyocarditis [25]. The authors assumed that the induction of interferons was a result of protein-protein interactions, mediated by a viral fusion protein GP67 (GP64) and corresponding cellular receptors, as antibodies to the fusion protein inhibit the induction of interferons.

In many works the recombinant baculoviruses, carrying the genes of different structural components of mammalian viruses, were studied as antigen-expressing immunovectors. The experiments on animal models demonstrated the induction of specific humoral immune response to the introduction1 of transducing baculoviruses with genes of pseudorabies virus glycoprotein B (which causes acute viral disease of the animal nervous system) [26], hemagglutinin of influenza virus [27], glycoprotein E2 of hepatitis C virus or carcinoembrional antigen (CEA) [28], surface-displayed protein E of Japanese encephalitis virus [29].

Special interest of researchers is attracted to the creation of living anti-influenza vaccines, elaboration of injection-free ways of their administration as well as to the technology of mass production of vaccine preparations on the basis of recombinant baculoviruses. A number of works showed baculovirus-mediated induction of systemic and mucous immune responses at intranasal and peroral introduction which makes them promising for gene therapy manipulations and creation of living low-toxic immune-vector vaccines.

Different groups of researchers demonstrated on the model systems high efficiency of anti-influenza vaccines based on recombinant baculoviruses with the hemagglutinin gene of avian influenza virus H5N1 [30, 31]. The advantage of vector recombinant baculoviruses, expressing hemagglutinin and exposing its molecules on the surface of a virus particle, is that sparingly soluble highly hydrophobic protein is presented at the virus display in native functional conformation, which in its turn promotes maximal specific immune response to the introduced antigen.

The encouraging results were obtained on a murine model while testing peroral introduction of recombinant baculovirus with hemagglutinin gene of H5N1 virus using immediate early strong promoter of white spot syndrome virus (WSSV). The result was 100% defense of mice against the lethal dose of highly pathogenic strain of avian influenza virus which is supported by a considerable level of specific serum immunoglobulins and mucous immunoglobulins IgA [32].

A high degree of mutational variability of influenza viruses (mainly it is related to either point or considerable modifications of amino acid sequences in the area of antigen determinants of hemagglutinating and neuroamidase subunits) determines the necessity of producing vaccine preparations against ever appearing new variants of the virus. This situation is aggravated when it is necessary to produce large volumes of new vaccine in a short period of time, taking into consideration the previous varieties of influenza virus and its functioning in the population. Every year the influenza virus changes with the prevalence of its other strains. Sequences of hemagglutinin of the majority of avian influenza viruses H5N1, circulating in recent years, fall into two genetic groups or clades. Due to high speed of mutation

of this virus each specific composition of the vaccine is maximum effective for about one year. The way out of this situation is the creation of polyvalent vaccines on the basis of new biotechnologies. The effort to do it using recombinant baculoviruses was reported in [33]. Having analyzed the primary structure of the main neutralizing epitopes of hemagglutinin of different lines of avian influenza virus, the authors selected three vaccine strains, covering all the neutralizing epitopes of hemagglutinin of known virus lines and exposed the hemagglutinins selected on the surface of recombinant baculoviruses. Thus, the polyvalent anti-influenza vaccine was created on the basis of recombinant baculovirus and baculophage technology. The testing of this vaccine, performed on mice, showed its high efficiency in the neutralization of viral infection while using six different genetic groups as a source of infection of avian influenza virus. At the same time monovalent baculovirus vaccine was efficient regarding homologous clade and two more genetically close groups.

It is noteworthy that both baculovirus *A. californica* and NPV *Bombyx mori* (BmNPV) were used as vectors. Chinese researchers constructed the recombinant virus Bmgp64HA, carrying hemagglutinin molecule of avian influenza virus on viral surface of the particle. The recombinant virus was replicated in pupa of *Bombyx mori*, peculiar bioreactors, and after extraction and purification it was used in experiments with monkeys and mice. It was shown that this virus keeps 50–67% of animals from influenza infection depending on the dose [34].

The capability of non-recombinant baculoviruses to induce a primary (innate) immune response at the level of activation of pro-inflammatory cytokines synthesis, demonstrated in the previous works, also attracted the attention of researchers. The induction of cellular immune response, mediated through the activation of the synthesis of a number of cytokines, TNF-b, IL1-b, IL1-g by the non-recombinant baculovirus in rat hepatocytes, was shown in the early work of authors [35].

The investigation [34] was developed in the previously mentioned research of Japanese scientists [27]. They revealed that introduction of both the recombinant baculovirus, expressing hemagglutinin gene of H1N1 influenza virus, and the baculovirus of wild type actively prevents the development of viral infection. At the same time it was observed that intranasal introduction of baculovirus was the most effective method. This observation along with the data about the activation of murine macrophages by the virus allowed the authors to assume possible mechanisms of induction of antiviral immune response by the baculovirus. The presence of mannose monosaccharide in the structure of fusion protein of the baculovirus GP64 results in close interaction with mannose receptors, participating in the formation of the immune response, on the surface of macrophages and dendritic cells, considerably present in lymphoid tissues of nasopharynx and lung tissues, thus inducing the synthesis of anti-inflammatory cytokines and antiviral response in them. Further on these authors proved that the interaction of baculovirus and dendritic cells of murine bone marrow enhances the expression of such anti-inflammatory cytokines as IL12, IL16, and IL-1в [36].

In this respect, rather interesting are the data obtained in experiments on mice. They show] that the activation of synthesis of interferons by baculovirus induces both humoral and cellular immune response, thus increasing the effect of introduced antigen. Therefore, the baculovirus shows the features of quite efficient adjuvant. Moreover, the authors state that even the presence of viral particles in preparations of recombinant proteins, obtained in baculovirus expression system, increases their immunizing power considerably [37]. Detailed study of the baculovirus action as an adjuvant allowed the authors to conclude that the viral fusion protein GP64 is most likely not a key factor in this process, but they do not exclude the participation of other viral components in the mentioned mechanism.

This conclusion is supported by Abe *et al*. [38], stating that the baculovirus-induced activation of the

immune system is most likely achieved via signalling pathway MyD88/TLR9. As the evidence, the authors present the results of their investigations, where the transfection of murine macrophages of RAW264.7 line by genome baculovirus DNA activates the synthesis of pro-inflammatory cytokine TNFб. Taking into account that baculovirus DNA contains a significant number of potentially active CpG-dinucleotides, and [that the] receptors Toll-like9 actively interact with non-methylated CpG-dinucleotides of bacterial DNA molecules, this conclusion could have been recognized as reasonable and logical. However, the same authors showed later [39] that along with the participation of Toll-like9 receptor signalling pathway in the initiation of interferon synthesis and chemokines, activated by them in immunocompetent cells, the induction of interferon in the cells of embryonal fibroblasts of mice occurs regardless of this signalling pathway and is mediated by interferon-regulatory factors IRF7/IRF3. The authors came to the conclusion that there might be a system of signalling pathways of induction of cellular adaptive and immune responses, dependent on the cell functional specialization.

In this respect noteworthy are the data of studies, dedicated to the response of mammalian cell genome to the introduction of baculovirus. For instance, it was shown that the transduction of HEK293 cells by nuclear polyhedrosis virus of *B. mori* results in considerable changing of the expression profile of about 20 cellular genes [40]. In this case the response of human MSC to the introduction of baculovirus AcNPV is even more expressed and accompanied by short-term deregulation of activity of about 800 genes, which take part in the functioning of five cellular signalling pathways, demonstrating the deviations in transcriptional activity for different genes towards both its induction and decrease. Also transient increase in the synthesis of two cytokines – IL6 and IL8 – was observed, and the total profile of expression of cytokine genes is quite different from that for specialized immune cells [41]. The authors revealed the activation of Toll-like3 signalling pathway by DNA-containing virus in human MSC which is rather nonspecific for this signalling pathway, normally induced by double-stranded viral and synthetic RNA. The result of experiment is quite intriguing and should be either confirmed or rejected by more detailed investigations.

The results of previous works on different aspects of interaction of baculoviruses with non-permissive cells and organisms, streamlined the development of investigations, dedicated to the improvement and application of recombinant baculoviruses as transducing vectors and efficient adjuvants for antigen peptides and proteins [42, 43].

The strategy of baculovirus vector improvement for the purposes of vaccine therapy consists in changing the surface of a viral particle in two ways: 1) genetic modifications of virus display for increasing efficiency of target transport, binding and introduction of recombinant baculovirus, protection of a viral particle from the effect of the complement in the course of introduction into the organism, and 2) exposing antigenic peptides and proteins on a viral particle by gene engineering or chemical method to increase specific immune response *in vivo* [44].

The system of genetic modification of baculovirus display, called “baculophage system”, is analogous to the bacteriophage display system. The modification is conducted by constructing a fused gene, including the sequence of baculovirus gene *gp64*, and heterologous protein or peptide under the control of a strong promoter of one of later baculovirus genes – polyhedrin gene [45–47].

A number of works were performed using genetic modifications of viral display by the abovedescribed method for the purposes of vaccine therapy. For instance, [the] recombinant baculovirus, carrying protein PbCSP of *Plasmodium berghe*i on the viral envelope, protected 60% of experimental mice against] further infection [48]. This result, though good enough,] did not satisfy the authors, and later they constructed a baculovirus with double expression system. The plasmodium antigen was inserted into the genome under the cytomegaloviral expression cassette and [was expressed on the surface of a viral particle in

insect cells] under the baculovirus promoter of polyhedrin gene. It enhanced specific humoral and cellular immune response at intramuscular introduction of immunovector [49].

The authors assumed that limited efficiency of traditional vaccines is caused by the induction of death of B-cells, genetic memory carriers, by plasmodium. A new recombinant baculovirus vector, constructed by them, presents a fragment of a surface protein PyMSP1 *Plasmodium yoelii* on the surface of a viral particle. At intranasal introduction of this vector] induces not only strong immune response, but also activates3 natural immune response to the infection [50].

It is noteworthy that [the] recombinant baculovirus with a double expression system was constructed and successfully applied in earlier similar investigations [51]. A special feature of the work was the usage of plasmodium, causing malaria in humans, which allowed the authors to compare the results, obtained *in vivo* on mice and *in vitro* on human cells. In these experiments the antigen was a surface protein CS of sporozoites with removed C-terminal sequence for anchor glycolipid GPI which, according to some data [52], interferes with the expression and immunogenicity of this protein. Out of three constructed vectors, the most efficient one was the baculovirus recombinant vector, combining both expression and presentation of CS antigen on the surface of a viral particle. This vector induces the maturing of CS-specific $CD4^+$- and $CD8^+$ T-cells in mice and activates the CS-specific $CD4^+$- and $CD8^+$ T-cell responses in the *in vitro* system quite efficiently while using human mononuclear cells from peripheral blood of healthy donors. The authors assumed that this vaccine vector may be effectively used for inhibition of hepatic stage of infection due to suppression of sporozoites division and elimination of infected hepatocytes.

In general, the abovementioned results of experiments with the application of recombinant and wild baculovirus vectors testify to their rather high efficiency in the induction of both adaptive and antigen-specific immune response[s].

Dendritic cells are known as highly specialized antigen-presenting cells, performing the functions of activation and modulation of T-cellular immune response. The initiation of adaptive immune response is highly dependent on the activation, functional maturing and migration of dendritic cells. In this respect, it is important that the interaction with baculovirus vector induces phenotypic transformation and functional maturing of dendritic cells, thus increasing their ability to stimulate T-cells and promoting the manifestation of strong adjuvant features [53, 54].

Given in this work, though far from complete, the list of publications presenting investigations on the influence of both recombinant and non-modified baculoviruses on the induction of cellular and humoral immune response, testifies to promising future of baculovirus application as low toxic prophylactic vaccines.

Baculovirus vectors in experimental cancer therapy. Due to a wide range of mammalian cells, which could be infected by baculovirus, including cancer cells of different origin, there is a possibility of applying recombinant baculoviruses in gene therapy of cancer. One of the first was the work, in which seven different recombinant baculoviruses with the gene of A-chain of diphtheria toxin were constructed. The most efficient promoter was found to be a cell-specific promoter of glial fibrillary acidic protein (GFAP) in the expression cassette, containing the enhancer of cytomegalovirus immediate early gene and inverted terminal repeats of adeno-associated virus. This combination provides a considerable increase in the level of expression of a target gene in comparison with low transcriptional activity of cell-specific promoter. This recombinant baculovirus actively transduces cancer cells and inhibits the growth of malignant brain glioma in rats [55].

The recombinant baculovirus, expressing the gene of telomerase-reverse rat transcriptase (TERT), was used as an immunovector in [56]. Contrary to many healthy tissues, the majority of malignant tumours express TERT to some extent, due to which this

enzyme is rather a good antigen for the induction of anti-tumour immune response. Indeed, the splenocytes, obtained from intramuscularly vaccinated mice, contain increased amount of telomerase-specific T-cells secreting IFN-г, $CD4^+$ predominantly. An increased activity of natural killer cells was also observed. The immunization of mice with this recombinant baculovirus results in the occurrence of anti-tumour immune response and revents them against the development of brain tumour at subsequent introduction of tumour cells.

In the opinion of authors of [57], the anti-tumour immune response, induced by the wild baculovirus, is conditioned by the activation of natural killer cells, which is mediated by antigen-presenting dendritic cells, transduced by baculovirus. PCR analysis showed that this baculovirus does not infect cells NK, T and NTK, but its genome was revealed in lymphocytes, accumulated in spleen and liver of animals, mainly at intravenous introduction. The model of mice, inoculated by melanoma B16 cells, presents persuasive proof of inhibition of metastases in liver by the virus and high survival of experimental animals. However, it was not clear if this anti-tumour effect is really induced by the introduction of baculovirus of acquired immunity proper.

To solve this question, the authors investigated the activation of tumour-specific cytotoxic lymphocytes, which, in the opinion of many researchers, plays an important role in the formation of anti-tumour response. The titre of specific antibodies was determined in mice, injected by B16 cells into spleen, with subsequent intravenous inoculation of baculovirus a day later. The baculovirus-treated mice were shown to have high level of tumour-specific antibodies in blood serum and the specific anti-B16-activity of cytotoxic lymphocytes was induced [58].

The baculovirus-activated anti-tumour mechanisms are assumed to consist in the initial induction of specific anti-tumour activity of NK-cells via initiation of synthesis of cytokines and NKG2D ligands, i.e. the molecules, the expression of which is launched at cancer transformation *and* infection by viral or bacterial pathogens. The acquired immunity is formed via induction of tumour-specific cytotoxic lymphocytes and antibodies [58].

One of the main purposes of cancer immunotherapy is to cause or enhance an immune response to tumour antigens in patients. To achieve this purpose several approaches were tested, including the application of quite powerful antigen-presenting cells ? dendritic cells, capable of efficient activation of T-cells. The presentation of peptides, the derivatives of tumour antigens on the dendritic cells surface, can stimulate strong anti-tumour immunity. Currently one of the new strategies in immune therapy of tumours is the application of recombinant virus vectors, coding tumour antigens and effectively expressing their stable levels on the surface of dendritic cells, which, in their turn, induce antigen-specific T-cellular response [59]. Owing to the efficient transduction of dendritic cells by the baculovirus the latter can be used as an inductor of anti-tumour response in the development of this strategy. Kitajima et al., actively developing this trend, use dendritic cells of bone marrow, transduced by the baculovirus, in model experiments on mice with lung carcinoma and melanoma; they demonstrated increased survival rates of experimental animals and considerable reduction of tumours [60].

Quite an important problem of gene therapy of carcinogenesis is search for and application of genes, the products of expression of which would be toxic for tumour cells, but would not damage normal cells. One of these genes is apoptin gene (apoptin is a small protein, obtained from chicken anemia virus, first name – VP3). The specificity of apoptin is inducing apoptosis in transformed and malignant human cells but not in normal ones [61, 62].

The application of a number of recombinant viruses (adenovirus, parvovirus, and poxvirus), containing the apoptin gene allowed obtaining quite promising results, testifying to efficient anti-tumour activity of recombinant vectors and selective effect of a target protein, expressed by them [63]. It is quite expected that the apoptin gene will be used for obtaining a

recombinant baculovirus and further study of its selective apoptotic effect on tumour cells. The recombinant baculovirus with apoptin gene under the promoter of cytomegalovirus efficiently induces the apoptosis *in vitro* in HepG2 and H22 cells and *in vivo* at intratumoral introduction in mice with hepatocarcinoma [64]. It was also shown that the most effective method was double introduction of the baculovector with apoptin gene in two- week interval, in such case the survival rate of experimental animals increased twice compared to the control carriers of tumour.

Quite a promising direction of gene therapy of cancer is considered to be the application of genes, coding suppressors of tumour growth. One of them is gene *Pdcd4*. The mechanism of its action is the binding of the product of gene expression with the factor of translation initiation A1, which prevents RNA binding to it. It was previously established that super expression of mRNA of this factor occurs in human melanoma cells [65].

Another way of increasing transduction efficiency in experiments was a usage of folate-pegylated recombinant baculovirus with the human gene *pdcd4* – F-P-Bac-Pdcd4. Intratumoral injection of such baculovirus results in considerable inhibition of tumour growth and induction of apoptosis of epithelial carcinoma cells, caused by subcutaneous introduction of the cell suspension of KB line (the cell line, originating from human nasopharyngeal carcinoma, which is a test of anti-tumour agents efficiency) [66].

Unfortunately, the application of gene therapy approach for inhibition of the development of cancer tumours with various genetic constructions, involving cytokine genes, suicidal genes or genes, inducing apoptosis, does not guarantee maximal therapeutic effect. As for the application of apoptotic genes, in our opinion, principally different vector systems are necessary, which would provide the delivery of suicidal genes or medicinal preparations directly to cancer cells and cause their apoptosis. Currently, the most promising candidates for this role are stem cells which have tropism to tumour cells and migrate to tumour tissues. The genetically modified stem cells, carrying therapeutic genes, may be used for the delivery of gene products to tumours and metastases.

A promising direction in the development of investigations in the field of gene therapy is the elaboration of new systems of delivery of target genes on the basis of elements of viral and non-viral vectors and nanoparticles, which should directly migrate to tumour cells, thus providing efficient transfer of target genes into the main bulk of these cells and prolonged expression of target genes without integration into their genome.

Therefore, it seems optimal to combine gene- and vaccine therapy to achieve maximal therapeutic effect. The efficiency of vector vaccine therapy may be considerably increased after overcoming the immune suppression in cancer patients, caused by both the presence of a large tumour and performance of preliminary chemo- and radiotherapy. Besides, due to heterogeneity of expression of tumour antigens in different patients and even in different cells of tumours and metastasis, the use of polyvalent vaccines on the basis of recombinant vectors seems to be optimal.

Obtaining additional information about the biology of baculoviruses and their interaction with unnatural hosts will undoubtedly promote the construction of highly effective recombinant vectors for their application in clinical practice. As for the future of recombinant baculoviruses, they most likely will be used as immunovector vaccines due to considerable cheapening and elaboration of modern technologies of scaling the process of accumulation of the modified virus and efficiency of application for preventing infectious diseases, influenza, in the first place. Their application as immunovaccines in treatment of cancer depends considerably on the elaboration of new efficient clinical protocols.

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Бакуловирусные вектора в экспериментапльной гено- и вакцинотерапии

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Резюме

Представлен краткий обзор данных литературы по целевому конструированию и исследованию свойств и эффективности применения в модельных системах in vivo рекомбинантных бакуловирусов. Проанализированы результаты экспериментов с использованием диких и рекомбинантных бакуловирусов в таких приоритетных областях биомедицины, как регенерация тканей, генотерапия рака, разработка вакцин против инфекционных заболеваний и злокачественных новообразований.

Ключевые слова: бакуловирус, гено- и иммуновектор, млекопитающие, система in vivo.

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Бакуловірусні вектори в експериментальній гено- та вакцинотерапії

Резюме

Представлено короткий огляд даних літератури стосовно цільового конструювання і дослідження властивостей та ефективності використано в модельних системах in vivo рекомбінантних бакуловірусів. Проаналізовано результати дослідів із застосуванням диких і рекомбінантних бакуловірусів у таких пріоритетних галузях сучасної біомедицини, як регенерація тканин, генотерапія раку, розробка вакцин проти інфекційних захворювань та злоякісних новоутворень.

Ключові слова: бакуловірус, гено- та імуновектор, ссавці, система in vivo.

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