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Development of Methods about Safe Application of Viral Strains with DNA-Genome as a Material for Design of New Molecular Vaccines against SARS-CoV-2/COVID-19

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ABSTRACT

Taking in consideration the risk of thrombs by SARS-CoV-2 Spike (S) protein and of amyloid brain plaques by its Nucleocapsid (N) protein, design of molecular (DNA, RNA and/or protein) vaccines against viral Envelope (E) protein or viral Membrane (M) protein, and boosting by siRNAs against virus genes, coding proteins S and N. Laboratory-incubated mammalian cells were transfected by recombinant gene construct. This vector and separate sub-populations of transfected cells, contained additional gene copy, demonstrated safety and capability for generation of adequate immune response *in vitro and in vivo*. Other mammalian *in vitro*-cellular cultures were inoculated with low infectious titers (high viral suspensions dilutions) of vaccine avipoxviral strains (Poxviridae family), freezed in the presence of cryo-protector Dimethylsulfoxide (DMSO), thawed and re-incubated. As a source of extra-cellular virus served the cultural fluids, and of intra-cellular-scraped cellular monolayers. The titers of the intra-cellular forms were significantly higher that these of the extra-cellular. Transfer of nucleotide (DNA and/or RNA) fragments from virus to cellular genome, and in opposite direction due to activated fusion on the influence of DMSO plus drastic temperature changes were suggested. A possibility about application of viruses with DNA and/or RNA-genome for production of molecular vaccines and of appropriate siRNAs was proposed. *In vitro*-incubated cultures of non-malignant mouse embryonic cells; of containing endogenous retrovirus mouse malignant myeloma cells, and of mixed cultures of both were lysed and subjected on ELISA for determination the antibodies presence and titers. A possibility for production of membrane receptor glycoproteins and antibodies/immunoglobulin's by non-myeloid and non-lymphoid cells in appropriate conditions was suggested. Because the produced antibodies are out of the germinative centers in the lymphoid tissues and organs, for escape of chronic inflammation,

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often leading to malignant transformation or degenerative changes, they should be controlled by small ions or molecules.

Keywords: Viral strains; Molecular vaccines; COVID-19; DNA genome; ELISA

INTRODUCTION

The potential of viruses as appropriate vectors for development of various therapeutic strategies, as well as for design of molecular (with DNA, RNA and/or protein nature) vaccines by substitution of appropriate cell nucleotide sequences has been proved [1-3]. These types of vaccines have shown many advantages compared with the attenuated, died and subunit vaccines. Both RNA and DNA viruses could be applied for this goal, but also bacteria plasmids and yeasts' genomes [4,5]. In all cases described, appropriate promoter gene should be inserted about the expression of the respective inserted gene of interest. The used initial vector constructs should contain specific restriction sites, and the respective DNA-fragment(s) of interest should be obtained by treatment with respective restriction enzymes (most often bacterial ends-nucleases). Other important components are the Inverted Terminal Repeats (ITRs). Possible modifications have been proved to be increased expression level of the foreign antigen by a change in the promoter or in the insertion site of the applied viral vector. These features are taken in consideration in design of different types of vaccines against Corona viruses (**Figure 1**) [6-9].

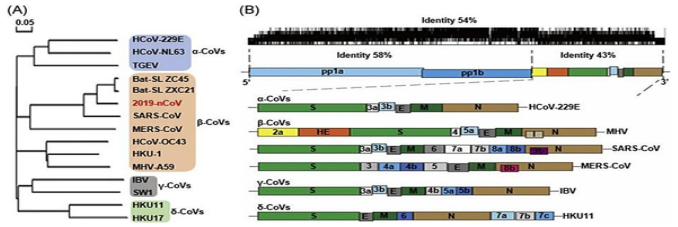

Figure 1: Genomic structure and phylogenetic tree of Coronaviruses: Phylogenetic tree of representative Coronaviruses, with SARS-CoV-2/COVID-19. A) Genome structure of four Coronavirus genera: Two long polypeptides 16 non-structural proteins have proceeded from Pp1a and pp1b represent. B) S, E, M and N are represented of the four structural proteins: Spike, envelope, membrane and nucleocapsid. HE: Hemagglutinin-Esterase. Viral names (HKU): Coronavirus (identified by Hong Kong University); HCoV-Human Coronavirus; IBV: Infectious Bronchitis Virus; MHV: Murine Hepatitis Virus; TGEV: Transmissible Gastroenteritis Virus.

The role of the cellular Angiotensin-Converting Enzyme 2 (ACE2) as the main target of SARS-CoV-2/COVID-19, as well as of the other members belonging to Coronaviridae family (as the agents, causing SARS and MERS) has been proved [10]. Each monomer of the trimeric Spike (S) glycoprotein consists of two subunits, S1 and S2, which allows binding to the ACE2 receptor and subsequent fusion of the viral and host cellular membranes. As an integral part in the activation and induction of a conformation changes in the S-protein–ACE2 receptor complex, which allows the further process of fusion between the virus particle and the host cell, has been characterized the enzyme serine protease (TMPRSS2) [11]. By penetration in the cell through the cellular receptor ACE2, the negative influence of these viruses, but even of their components, on the enzyme functions should also be taken in consideration as underlining in many symptoms of the infections, caused by them, including injured functions of many important anatomic organs [12,13]. Additionally, the role of Renin Angiotensin Aldosterone System (RAAS) has been proved as key in the regulation of the systemic blood pressure and renal function [14]. Therefore, methods and strategies for improving and regulation of their functions should also be developed [15]. Some differences in the arrangement of the Envelope (E), Membrane (M) and Nucleoprotein (N) among SARS-CoV-2, SARS-CoV and MERS-CoV have been shown (**Figure 2**) [16,17]. Thus, together with development of methods for activation of adequate antiviral immunity, strategies for suppression of key viral genes (as genes, responsible about the virus penetration in the cell, for the virus replication, but also the two processes) in the virus RNA-genome should also be developed (**Figure 3**) [18].

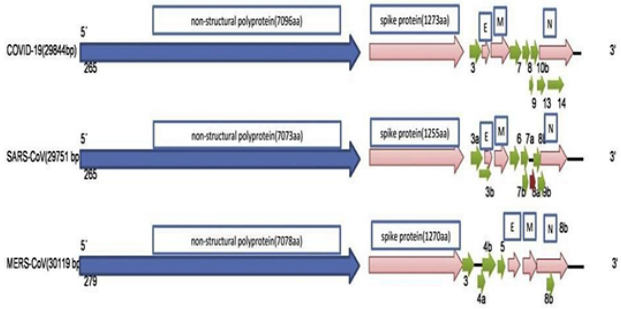

Figure 2: Differences in the arrangement of the Envelope (E), Membrane (M) and Nucleoprotein (N) among SARS-CoV-2, SARS-CoV and MERS-CoV.

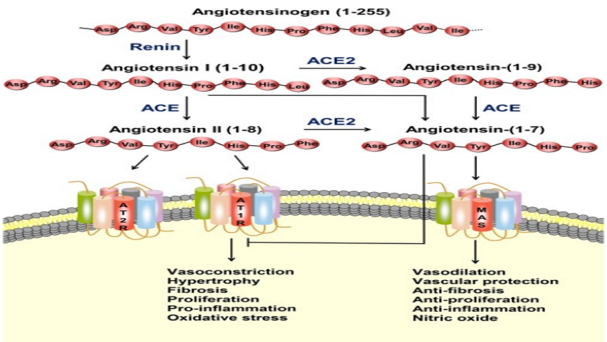

Figure 3: General illustration of the Renin-Angiotensin System (RAS) and ACE2/angiotensin-(1– 7)/MAS axis.

Renin is a protease, which converts angiotensinogen to Ang-I, which is subsequently converted to Ang-II by Angiotensin-Converting Enzyme (ACE). Ang-II is able to bind to the Angiotensin Type 1 Receptor (AT1R) to perform functions as vasoconstriction, hypertrophy, fibrosis, proliferation, inflammation and oxidative stress. ACE2 can covert Ang-I and Ang-II to angiotensin-(1–7). Angiotensin-(1–7) binds to the

MAS receptor to exert actions of vasodilation, vascular protection, anti-fibrosis, anti-proliferation, and anti-inflammation. Ang-II can also bind to the Angiotensin Type 2 Receptor (AT2R) to counteract the aforementioned effects mediated by AT1R (**Figures 3-5**) [19].

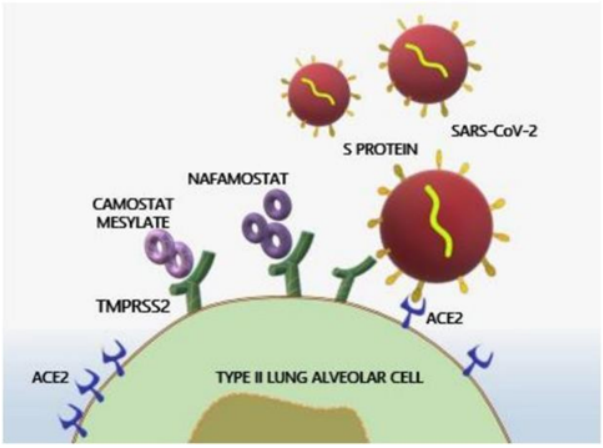

Figure 4: SARS-CoV-2 attachment and fusion machinery on human host cells.

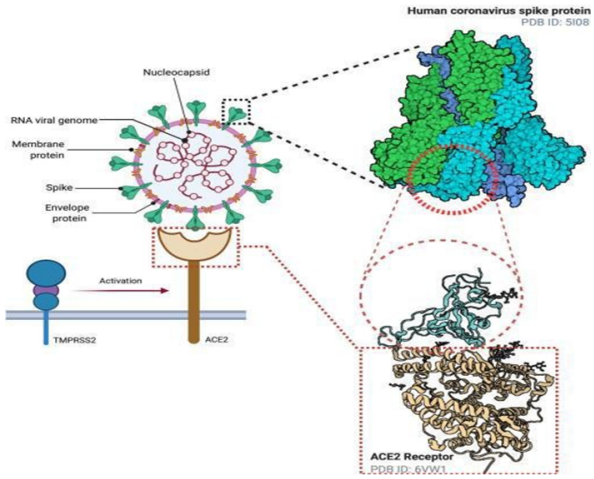

Figure 5: General illustration of the mechanism, by which SARS-CoV-2 penetrates in the cell by its Spike (S) protein through ACE2 cellular membrane receptor.

Vectors, based on adenovirus genome (Adenoviridae family), usually contain non-coding DNA, which is necessary to be ensured genome stability [20]. The early regions have been noted as E1–E4 along with the full-length major late transcript, followed by the late L1–L5 transcripts, produced through alternative splicing. Transcripts, encoding pIX, IVa2, and VA RNA I and II, are also shown. ITRs serve as origins of replication, and Ψ is the packaging sequence. When the early region E1 in the Ad vector is deleted, the inserted gene of interest normally replaces deletion E1 region and its expression is driven by a heterologous promoter. Most of these vectors have also been characterized with removed/deleted E3 region, but this region is not essential for the viral replication and allows insertion of a ~8 kb foreign expression cassette (**Figure 6**).

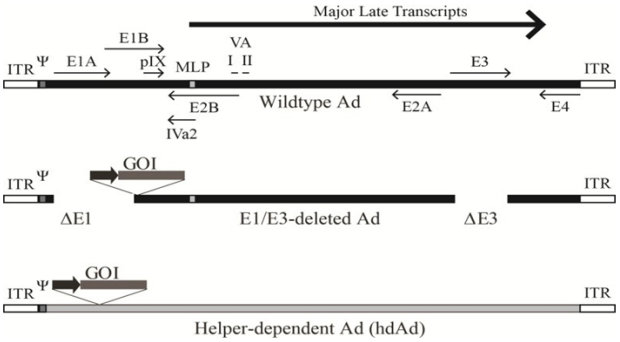

Figure 6: General presentation of the adenovirus genome and various adenoviral vectors (top panel).

The early regions are noted as E1–E4 along with the full-length major late transcript from which the late L1–L5 transcripts are produced through alternative splicing. Transcripts, encoding pIX, IVa2 and VA RNA I and II, are also shown. ITR represents the inverted terminal repeats that serve as origins of replication and Ψ is the packaging sequence. A typical early region (E1) deleted Ad vector is shown. The gene of interest (GOI) normally replaces the E1 deletion and its expression is driven by a heterologous promoter (Middle panel-dark arrow). Most of these vectors also have the E3 region removed, but it is not essential for viral replication and allows for insertion of a~8 kb foreign expression cassette (bottom panel). All the DNA encoding viral proteins are removed, only the ITRs and packaging sequence are required. These vectors usually have non-coding stuffer DNA (grey) to ensure the genome is stable. MLP-Major Late Promoter.

Most of the features of the Adeno Associated Virus (AAV) (Parvoviridae family) have identified it as highly desirable as a therapeutic gene carrier, mainly because of the non-pathogenic nature of its wild-type and its ability to infect non-dividing, terminally differentiated cells, as well as because of its sustained transgene expression *in vivo*. However, even when all the DNA-encoding viral proteins are removed, only the ITRs and packaging sequence are required (**Figure 7**).

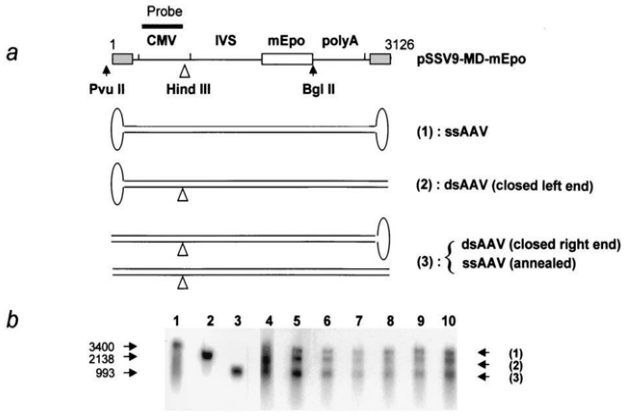

Figure 7: Southern blot analysis of the vector DNA after alkaline gel electrophoresis.

Positions of restriction sites and probe (CMV promoter) in pSSV9-MDmEpo. The different species expected to be detected in the analysis are represented below. Three

different fragments are expected upon HindIII digestion of these forms: (1) Undigested ssDNA, (2) Fragment from closed-left-end ds species (ds forms resulting from the synthesis of positive strand), and (3) Fragment either from re-annealed ds species or from closed-right-end species (ds forms resulting from synthesis of negative strand). IVS, second intron from the human *B-globin* gene (A); Southern blot following alkaline gel electrophoresis of HindIII-digested total muscle DNA (4 mg) from animals sacrificed on day 1 (lanes 5 and 6 correspond to two different DNA samples from the same injected muscle split prior to the extraction process, and lane 7 corresponds to a second animal), day 2 (one animal, lane 8) and day 3 (two animals, lanes 9 and 10). Lane 4 contains DNA from 293 cells coinfected with AAV-MD-mEpo and a helper adenovirus as a control for second strand synthesis. Lanes 1 to 3: Purified DNA from rAAV green fluorescent protein neo particles (lane 1), PvuII/BglII fragment from pSSV9-MD-mEpo (lane 2), and PvuII/HindIII fragment of pSSV9-MD-mEpo (lane 3) (B). Sizes in bases are indicated on the left. DNA-genome of the poxviruses (Poxviridae family) has been characterized among the most appropriate and applicable materials for production of gene-engineering vaccines and vectors for transfer of nucleotide fragments. Its expression could be provided by integration of appropriate promoter gene, which should be located out of the tk locus in the virus DNA-genome because of the proved necessity of intact tk gene. Most often production of recombinant vectors and vaccines, based on the poxviral DNA-genome, become by homologous recombination or by a direct molecular cloning. Different methods and techniques for maximally safe application of the received recombinant constructs on both cellular and organism levels should be developed, which depends on the type of the respective cells and organisms, as well as of the respective biological species. In this relation, some of the necessary characteristics are: Application of attenuated vaccine viral strains; appropriate initial infection titer (concentration of infections particles in minimal volume, respectively), as well as heterologous strains of the respective cells, tissues, organs or organisms for instance, application of homologous avian viral and bacterial strains in work with mammals and/or cells with mammalian origin. In this direction, the potential of avian pox viruses as appropriate vectors for development of various therapeutic strategies with mammals and mammalian cells has been proved in many literature findings.

So, the main goal of the presented study is directed to development of novel safe methods about application of viruses as vectors for design of new molecular anti-SARS-CoV-2/COVID-19 vaccines against other virus proteins (different of viral proteins S and N), with simultaneous boosting by appropriate siRNAs against virus genes, coding viral S and N proteins, and availability of adequate immune reaction.

MATERIALS AND METHODS

Insertion of Nucleotide Fragments by Previously Designed Recombinant DNA Viral Vectors

The obligatory components of the developed recombinant DNA-genome from Adeno-Associated Virus (AAV)(Parvoviridae family), besides the respective gene of interest, were also promoter for gene, coding eukaryotic elongation factor 1-alpha (EF1-α) and previously isolated from bacterial DNA-plasmid marker gene, providing resistance to Neomycin antibiotic. Genomic assay was made by standard Polymerase Chain Reaction (PCR), and expression of the respective inserted gene by Reverse Transcriptase PCR (PR-PCR), respectively, both by application and of specific primers and followed by electrophoresis in 1% agarose gel. A sub-population of *in vitro* incubated mouse Embryonic Stem Cells (mESCs) was transfected with the so received recombinant DNA-constructs. The selection of mESCs, containing additionally-inserted copy of the respective gene of interest was made on the basis of the presence of the near localized gene, determining the resistance of neomycin, by incubation in the presence of its synthetic analog G-418. The cells were seeded at initial dilution 1×10^6 cells/ml cultural fluid, incubated at 37°C in thermostat with 5% CO_2 and 95% air humidity, and observed at 24 hours intervals, by inverted light microscope Televal, supplied by mega-pixel CCD-camera as fixed slides, prepared by treatment with ethanol after previous washing by Phosphate Buffered Saline (PBS sigma) and subsequent staining by Giemsa dye (Sigma).

Insertion of Nucleotide Fragments by Activation of Fusion Process

Low initial infectious virus titers (high initial dilutions, respectively) of the initial suspensions of heterologous for mammals and mammalian cells probably attenuated by many passages vaccine avipoxviras strains (Poxviridae family) were applied. The so inoculated semi-confluent mono-layers of embryonic mammalian cells were then freezed at −80°C in the presence of cryo-protector Dimethylsulfoxide (DMSO sigma), subsequently thawed and re-incubated in fresh cultural fluid. After washing with PBS (sigma), the monolayers of the inoculated mammalian cells were scraped-off and subsequently used as a source of intra-cellular virus forms of the vaccine viral strains. Cultural fluids of the incubated and inoculated mammalian cells served as a source of the extra-cellular forms of the same vaccine strains. *De novo*-seeded cultures of the same mammalian cells were then inoculated with the so prepared intra and extra-cellular forms of each one of the two vaccine avipoxviras strains. All cell cultures were seeded at initial dilution 1×10^6 cells/ml cultural fluid, incubated at 37°C in thermostat with 5% CO_2 and 95% air humidity, and observed at 24 hours intervals, by inverted light microscope Televal, supplied by mega-pixel CCD-camera, as native preparations.

Experimental *In vivo* Models

Because of the proved reliability of the blood picture for the influence of various internal and external factors on the health parameters and immunological status *in vivo*, smears from peripheral blood of the tail vein of experimental mice from Balb/c line were prepared. The animals were separated into three groups: Non-inoculated controls; inoculated with recombinant DNA-construct/vector, containing additionally-inserted gene copy, and inoculated with suspension of transfected by the same recombinant vector mESCs, containing additionally-inserted copy of the same gene. Fixed light-microscopy slides, stained by Hematoxillin and Eosin (H/E) technique, were prepared.

Western-Blot (Immunoblot) Assay

Aliquots from the lisates of proteins, produced by the "donor" recombinant DNA-viral vector, from the "recipient" recombinant DNA-construct, but also from lysate of transfected cultivated mESCs, containing additionally-inserted copy of the same gene of interest (after previous trypsinization, resuspendation and centrifugation) were harvested, washed, in PBS and treated with lysis buffer on ice. The extracts with protein content adjusted to 10 µg/10 µl were subjected on Polyacrylamide Gel electrophoresis (SDS-PAGE). After washing the gel with distilled water, it was transferred to a methanol-pretreated P-0.45 polyvinylidene difluoride membrane (GE healthcare life science, Freiburg, Germany) by application of transfer buffer (100 ml 10 × TBS: 24.2 g Tris-HCL; 5.6 g Tris base; 80 g NaCl, pH 7.6). The membrane was then treated with blocking buffer (5% bovine serum albumin in 0.1% Tween/1 × TBS) on shaking machine for 1 hours-1.5 hours, and then with washing buffer (0.1% Tween/1 × TBS) for 10 minutes. After overnight incubation of the membrane with primary antibody at 40°C, it was then washed in washing buffer on shaking machine 3 times for 45 minutes and consequently in blocking buffer. The second antibody, diluted (1:2000) in 2% dry nonfat milk, was applied for 3 hours-4 hours at room temperature on shaking machine. The data were processed by application of Image software.

ELISA Protocol

Mouse embryonic fibroblasts from line 3T3 were incubated Dulbecco's modified Minimal Essential Medium (DMEM)(Sigma-Aldrich), supplemented with 10% Fetal Calf Serum (FCS) (Sigma-Aldrich), 100 IU/ml penicillin (Sigma-Aldrich) and 100 µg/ml streptomycin (Sigma-Aldrich). Also, suspension cultures of mouse malignant myeloma cells, containing endogenous retrovirus (Retroviridae family, possessing RNA-genome), were incubated in analogically supplemented medium RPMI 1640. Separate sub-population of non-malignant fibroblasts was pre-incubated in cultural fluid from previously incubated in it containing endogenous retrovirus malignant myeloma cells, after previous centrifugation and filtration. According to the literature data, by retroviral vector has been inserted bacterial gene *lacZ*, coding enzyme beta-galactosidase, which has been subsequently introduced in Dendritic Cells (DCs). The prepared cultures were put at 37°C in thermostat with 5% CO_2 and 95% air humidity, at initial dilution 1 × 10^6 cells/ml cultural fluids, and subsequently lysed by treatment of previously prepared cell suspensions with 10% Tri-Chloroacetic Acid (Cl_3CCOOH) and 0.48 M solution of K_3PO_4. For determination of the titers of anti ganglioside antibodies, 1000 ng of the respective ganglioside (Sigma) in 100 ml methanol were pipetted into microtiter plate wells (96-well plaques), containing the tested biological material. Subsequently, the wells were air-dried and blocked with BSA-PBS (Sigma) (1% bovine serum albumin in PBS) for 1 hour. After six-fold washing with PBS, 100 µl of each one of the prepared lysates from the cellular cultures, described above, diluted 1: 20 to 1:5000 in PBS were added to each well and incubated overnight. Then the plaques were washed six-fold with PBS. Binding was detected by following 2 hours incubation period with BSAPBS (Sigma) diluted (1/3200) peroxidase-conjugated goat antihuman IgG antibodies (Bul bio ltd., NCIPD, Sofia). All the incubations were performed at 40°C. The plates were then washed six times with PBS. Color development was achieved in a substrate solution, previously prepared from 15 mM O-Phenylenediamine and 0.015% H_2O_2 in 0.1 M sodium acetate buffer (0.2 M CH_3COONa/0.2 M CH_3COOH; pH 5.0) at 20°C. The reaction was stopped after 30 minutes with 50 ml of 1N H_2SO_4, and the Optical Density (OD) was assessed spectrometric ally at 490 nm on ELISA-reader (TECAN TM, Sunrise, and Austria). Non-specific antibody bindings (OD value in a well not containing the specific molecule in the respective probe) were subtracted for each measurement. The data were considered strongly positive, when the mean OD exceeded 2 ± SD (Standard Deviation), compared with the controls. The standard error of mean varied between ± 0.1 and ± 0.2. For the best reliability the procedure was performed three times.

RESULTS

Presence and expression of the inserted copy of the respective gene of interest in separate sub-populations of mammalian cells, transfected by based on AAV DNA-genome recombinant DNA-constructs, containing it, was proved by standard PCR and RT-PCR, respectively, as well as by the results from the performed western-blot (immunoblot) assay (**Figures 8 and 9**).

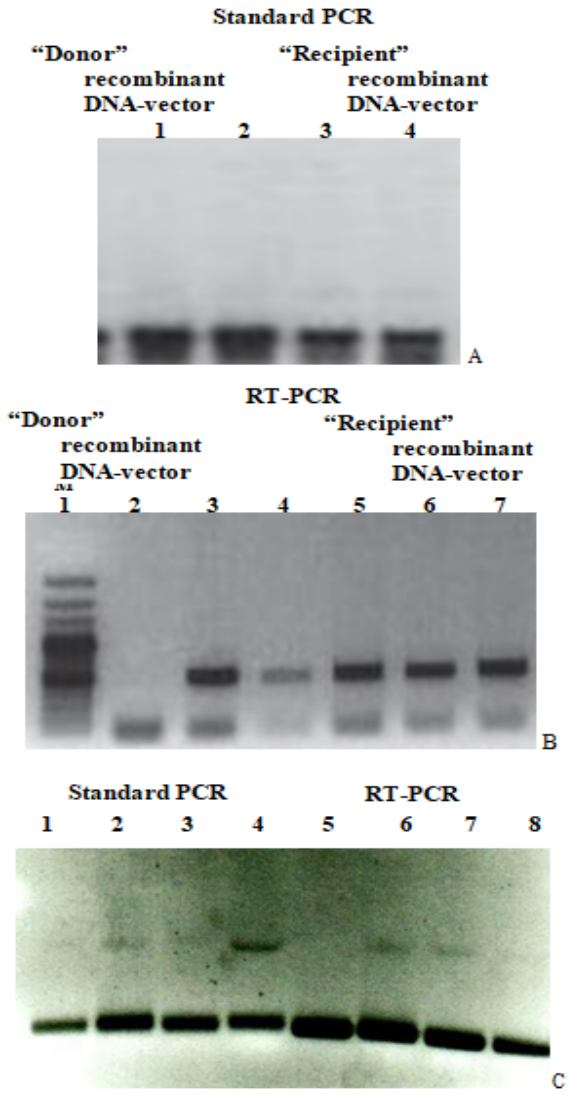

Figure 8: Agarose gel electrophoresis for prove of the presence of additionally-inserted gene copy in the applied for transfection based on AAV recombinant DNA-construct and for prove of its expression.

By standard PCR (A) and RT-PCR (B), respectively, and in the "donor" recombinant viral DNA-vector (A–lanes 1 and 2; B-lanes 3 and 5) and "recipient" recombinant DNA-construct (A–lanes 3 and 4; B–lanes 6 and 7); lane 1-marker (M); as well as for prove of presence of additionally-inserted gene copy in transfected mESCs (C), by standard PCR (C-lanes 2 and 4) and RT-PCR (C-lanes 6 and 7), by application of specific primers, complementary to the used for transfection recombinant DNA-construct and thus, to the inserted in them additional gene copy e of additionally-inserted gene copy in transfected mESCs, by standard PCR (C-lanes 1 and 4) and RT-PCR (C-lanes 6 and 7), by application of specific primers, complementary to the used for transfection recombinant DNA-construct and thus, to the inserted in them additional gene copy.

Figure 9: Western-blot assay by electrophoresis on polyacrylamide gel for prove the protein product of the tested gene of interest: Lane 1–from transfected MESCS, containing additionally-inserted copy of the gene of interest; lane 3–from the "donor" recombinant viral DNA-vector; lane 5–from the "recipient" recombinant DNA-construct.

Although significant differences between the blood pictures from the experimental mice of the three groups were not observed increased number of cells, particularly of different types of white blood cells in the blood smears of the rodents, inoculated with recombinant DNA-construct and of the animals, inoculated with suspension of transfected with the same vector cells, containing additionally-inserted copy of the gene, compared with the non-inoculated controls. These data suggested the safety of the applied recombinant viral vector and of the transfected with it cells, containing additionally-inserted gene, but on the other hand, generation of adequate immune response of the organism, depending of the respective factor influence (**Figure 10**).

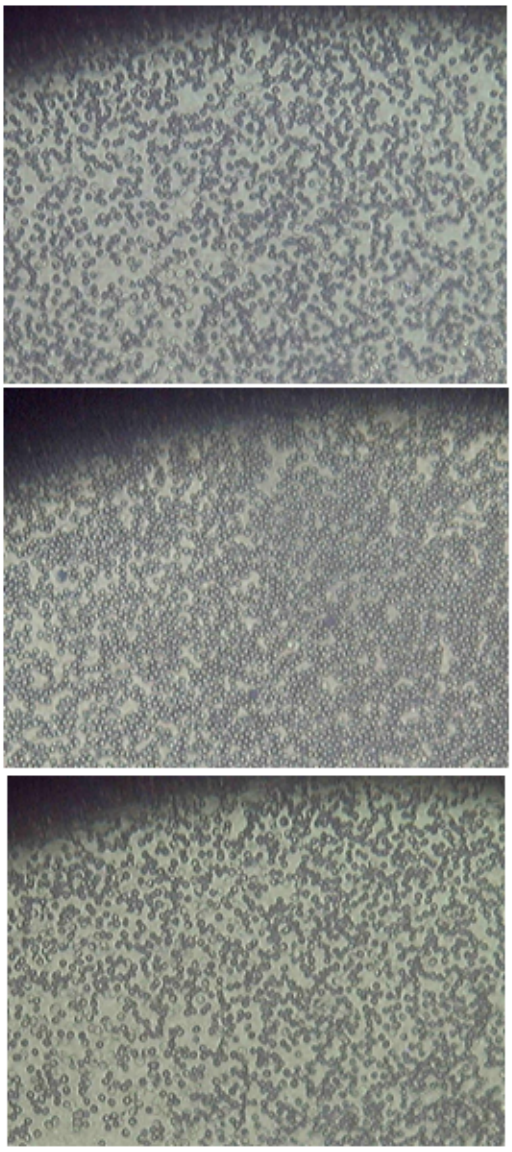

Figure 10: Peripheral blood smears from Balb/c experimental adult mice: A) Non-inoculated control; B) Inoculated with recombinant DNA vector, containing inserted copy of gene of interest; C) Inoculated with suspension of transfected mESCs, containing additional copy of gene of interest (fixed light microscopy preparations, stained by H/E, magnification: 100X).

In the blood picture from mouse, inoculated with recombinant DNA vector, containing inserted copy of the gene of interest, cellular differentiation of white blood cell directions from lymphocyte direction monocyte/macrophage lineages was observed. The blood picture from mouse, inoculated with suspension of transfected mESCs, containing additionally-inserted copy of the same gene was neares to this from the non-inoculated control, which was a prove about normal differentiation to the different blood cell lineages in both presence and absence of these transfected cells.

The observed in all cases higher titers of the intra-cellular forms of both vaccine viral strains compared to their extra-cellular forms (**Figures 11 and 12**) could suggest a presence of the respective strain in various sub-forms in the cell, including as a pro-virus.

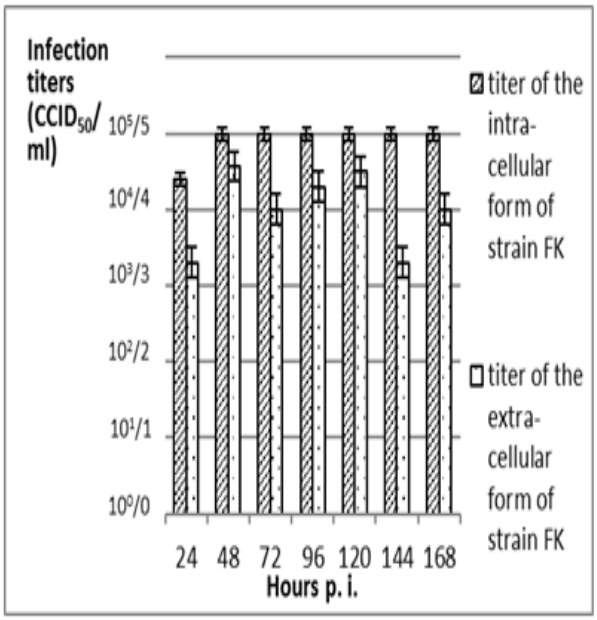

Figure 11: Infectious titers of intra and extra-cellular forms of fowl pox vaccine viral strain FK on laboratory incubated mammalian cells, P<0.01.

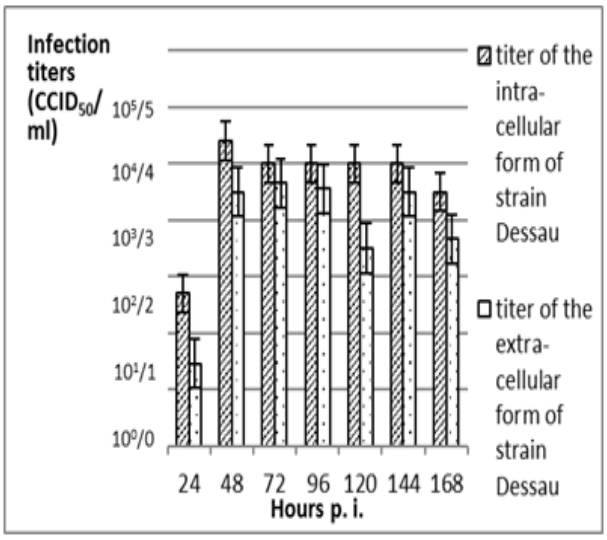

Figure 12: Infectious titers of intra and extra-cellular forms of pigeon pox vaccine viral strain Dessau on laboratory incubated mammalian cells, P<0.01.

The question, which arose, was: What makes the cells as acceptable about external inviders as viruses, malignant cells/ antigens, etc.? In most cases, higher titers of the anti-ganglioside antibodies in the lysates from the cultures of malignant myeloma cells and from the mixed cultures of fibroblasts, pre-incubated in cultural fluid from the malignant cells, compared to their titers in the lysate from the non-malignant 3T3 fibroblasts, were assessed (**Figure 13**).

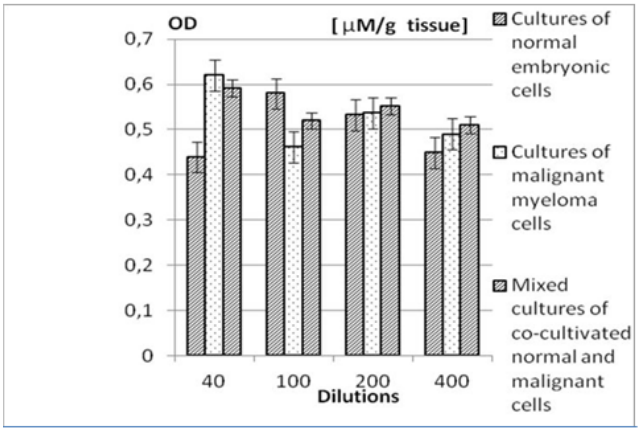

Figure 13: Average titers of anti-ganglioside antibodies in extracts from *in vitro* incubated cultures of non-malignant 3T3 mouse embryonic fibroblasts, of containing endogenous retrovirus mouse malignant myeloma cells, as well as from mixed cultures of both cellular types. OD–Optical Density, SD between ± 0.1 and ± 0.2, P<0.05.

DISCUSSION

According to the performed standard PCR and RT-PCT with subsequent electrophoresis, but also to the performed Western-blot (Immunoblot) assay with subsequent SDS-PAGE, in both "donor" and "recipient", as well as in the transfected cells was detected the presence of additionally-inserted copy of the respective gene of interest and its expression, respectively. This study was performed for investigation on the possibility about insertion of foreign genes in the DNA-genome of the adeno associated viruses and thus, the possibility about application of these viruses for various gene-engineering manipulations in the concrete case, about production of molecular vaccines and siRNAs. The data obtained additionally confirm the usability of the applied methods. The established higher titers of the intra-cellular forms of both vaccine avipoxviral strains, compared with these of their extra-cellular forms from the 24th to the 168th hour P.I. could be explained with eventual existence of the intra-cellular forms in several different variations, including as pro-viruses. Additionally, the proved in the scientific literature changed properties of many membrane molecules on the influence of DMSO and of the drastic temperature changes, but also with the influence of many inter-molecular interactions, leading to activated fussion processes, was suggested. Thus, a possibility for transfer of nucleotide (DNA and/or RNA) fragments from virus to cellular genome, as well as in the opposite direction (from cellular to viral genome) in these conditions could be proposed. In this way, a possibility about development of methods for production of molecular vaccines, as well as of appropriate siRNAs on the basis of poxviruses by appropriate application and processing could be suggested. Several main mechanisms of malignant cellular transformation by endogenous retroviruses have been proved: By *in vivo* infection of newborn mice, by xenograft inbred rodents, as well as by inoculation of *in vitro* incubated cells. The results obtained confirmed a possibility for production of appropriate molecular vaccines and RNAs by exchanges of nucleotide sequences between viral and cellular genomes. In this way, a possibility about application of retroviruses for production of molecular vaccines and siRNAs was confirmed. On the other hand, in all cases the current results confirmed the literature findings about a possibility for expression of immunoglobulin's/antibodies and membrane receptor glycoproteins by non-myeloid and non-lymphoid cellular types in appropriate conditions, as for instance, presence of malignant cells or malignant antigens, of viruses or viral antigens, as well as appropriate cytokines, immunomodulators, etc. In this way, the current data suggested a possibility about development of novel prophylactic, diagnostic and therapeutic strategies. However, because the so produced antibodies are out of the germinative centers of the specialized lymphoid tissues and organs, the control of their functions by is of key importance, for escape of chronic inflammatory process, which could lead to malignant transformations or to degenerative changes. For providing and support of this control have been found to be responsible small ions and molecules as ganglioside, by direct participation, but also by indirect influence on various inter-molecular interactions and cascade regulatory pathways.

CONCLUSION

In the current research paper was taken in consideration the proved activated formation of thrombs on the influence of Spike (S) protein of virus strain SARS-CoV-2, as well as of the proved activated formations of observed in different dementias protein plaques in the brain on the influence of Nucleocapsid (N) protein of the same virus. In this relation, possibilities about design of molecular (DNA, RNA and/or protein) vaccines against other virus proteins, different of proteins S and N, for escape of the risks, mentioned above, were investigated, but also of specific siRNAs against the virus genes, coding viral proteins S and N. In this connection, possibilities for application of viruses, possessing DNA and RNA-genomes as appropriate vectors about transfer of nucleotide sequences in the cell, between to each other, as well as from cellular genome to virus strains, were developed and tested, by development and application of techniques about activation the fusion process as influence of organic detergent (DMSO in the concrete case) and of drastic temperature changes. In all cases was shown a possibility about application of viruses, possessing DNA and/or RNA-genome for production of molecular vaccines, as well as of appropriate RNAs. Additionally, in support with the literature data, a possibility for production of membrane receptor glycoproteins and of immunoglobulins/antibodies by non-myeloid and non-lymphoid cellular types in appropriate conditions was suggested, which proposed a possibility about development of novel diagnostic, prophylactic and therapeutic approaches. Further studies are necessary to be performed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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