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Development of Techniques about Production of Recombinant vaccines and cells with Activated Immunogenic potential

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Research Article

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Abstract

Methods about derivation of myeloid-like and lymphoid-like cells from normal embryonic fibroblasts were developed and tested. Balb/c mice normal mouse embryonic cells from line 3T3 were co-cultivated with mouse malignant myeloma cells, containing inserted. Murine Leukemia Virus (MuLV) (with RNA-genome, *Retroviridae* family). For this goal, separate sub-population of normal mouse embryonic fibroblasts were pre-incubated in cultural fluid, supplemented by previous incubation of myeloma cells in it, after subsequent centrifugation and filtration. Other sub-populations of 3T3 cells were co-cultivated with myeloma cells, by addition of cultural fluids plus cellular suspensions of both types. Subsequently, all mixed cultures were freezed after addition of cryo-protector Dimethylsufoxide (DMSO), subsequently thawed and re-incubated in standard laboratory conditions. In the cultures, pre-incubated in cultural fluid, supplemented by previous incubation of mouse malignant myeloma cells, cells in different stages of myeloid/phagocyte and lymphoid/plasmatic cell differentiation were observed, but in addition of suspensions of cells from both types, appearance of hybrid cells of myeloid-like and phagocyte-like, as well as of lymphoid-like and plasmatic cells-like with mouse malignant myeloma cells were noted. The established changes could be explained with the eventual existence of capable to differentiate to various lineages stem-like cellular sub-populations in the general 3T3 cell line. Also, activated fusion between different cells, as well as between cells and viral particles on the influence of DMSO and of the drastic temperature changes was proposed, which could also lead to transfer of nucleotide sequences. On this principle, a possibility for production of recombinant viral vaccines by exchange of nucleotide sequences between cells and viral particles could be suggested. Furthermore, in confirmation of the literature findings, a capability of non-myeloid and non-lymphoid cells to produce membrane receptor glycoproteins was proposed.

Keywords: Myeloid/Phagocyte; Lymphoid/Plasmatic Cells; Phenotypic Characteristics; Activated Fusion; Hybrid Cells; Transfer of Nucleotide Sequences

Introduction

The possibility about *in vitro*-incubation of viruses in cell cultures, with the aim for development of both viral recombinants for immunotherapeutic procedures, is widely investigated. As such tools can be used for DNA- and RNA-viruses, as well as bacterial plasmids, yeasts and other eukaryotic cell types [1-6]. Besides the respective gene(s) of interest, appropriate promoter gene should be inserted, necessary to provide its expression. As the most often used marker genes are these, which code color proteins (fluorescent protein, as GFP, YFP, RFP) or products, participating in a color reaction (enzymes luciferase or timidinkinase), or gene, determining antibiotic resistance. The described gene sequences should be connected by

treatment with specific ligases, but the used initial vector constructs should contain specific restriction sites, which is necessary the withed DNA-fragments of interest be obtained by treatment with respective restriction enzymes (most often bacterial endo-nucleases). Other important components are the reverted end DNA-repeats. Different modifications by changes in the promoter, but also in the insertion site, as well as in the target vector repeats in fragments, expressing proteins with immunomodulator functions, have been proven to be possible. In this way could be inserted genes, coding cell receptors, cytokines, enzymes, complement activators, activators and/or inhibitors of apoptosis, surface antigens, tumor markers, etc. Polymerase chain reaction (PCR) of the received construction should then be performed by use of oligonucleotide primers for insurance of respective restriction sites – *SfiI*-restriction site on the 5'-end, and *RsrII*-restriction site on the 3'-end of the PCR-product, respectively, obtained by digestion with respective restriction enzymes (bacterial restrictases, in particular endonucleases), bound to the respective early or late promoter in the viral genome or plasmid DNA, but also from yeasts or other types of eukaryotic cells [3,6].

In this direction, the main goal of the current research paper was connected with development of *in vitro*-methods about universal immunity activation.

Materials and Methods

Normal 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 U/ml penicillin (Sigma-Aldrich) and 100 μg/ml streptomycin (Sigma-Aldrich). Also, suspension cultures of mouse malignant myeloma cells, containing inserted Murine Leukemia Virus (MuLV) (*Retroviridae* family, with RNA-genome) were incubated in analogically supplemented medium RPMI 1640. Mixed cultures were also prepared and analogically proceeded, by pre-incubation of sub-populations of normal mouse embryonic fibroblasts in cultural fluid, supplemented by previous incubation of mouse malignant myeloma cells in it, subsequently centrifuged and filtered, but also by addition of cultural fluids plus suspensions of cells from both types. All cultures were cultivated at 37^0C in incubator with 5% CO_2 and 95% air humidification. All cells were observed by inverted light microscope (Leica). Sub-population of 3T3 mouse embryonic fibroblasts from line were incubated in supplemented cultural fluids after previous co-cultivation with mouse malignant myeloma cells, and another sub-group was co-cultivated with sub-population of mouse malignant myeloma cells (cultural fluids plus suspensions of both cellular types). Sub-populations of both types of mixed cultures, described above, were then freezed in the presence of cryo-protector Dimethylsulfoxide (DMSO), subsequently thawed and re-cultivated in fresh medium in the standard conditions, described above.

Results

In co-cultivation of partially differentiated normal mouse embryonic fibroblasts from line 3T3 with mouse malignant myeloma cells, signs of early stages of initial myeloid-like and lymphoid-like cellular differentiation were noted (Figure 1A,B).

A B C

Figure 1: A- control semi-confluent monolayer of 3T3 normal mouse embryonic fibroblasts; B – incubated in cultural fluid from mouse malignant myeloma cells (eventually containing nucleotide sequences from the transforming vector), freezed in the presence of DMSO, thawed and re-incubated. Signs of initial myeloid and phagocyte, as well as of lymphoid and plasmatic cells phenotype, respectively, could be noted; C – co-cultivated with mouse malignant myeloma cells, freezed in the presence of DMSO, thawed and re-incubated. Appearance of hybrid cells between myeloid-like progenitors and myeloma cells and between lymphoid-like progenitors and malignant myeloma cells, respectively, could be seen, Fixed light-microscopy preparations, stained by Giemsa-dye, magnification: 100X.

In freezing of incubated in supplemented by previous cultivation of mouse malignant myeloma cells cultural fluid 3T3 mouse embryonic fibroblasts in the presence of DMSO, followed by thawing and re-incubation, appearance of myeloid-like and phagocyte-like cells, as well as such with lymphoid and plasmatic cells phenotypes were noted (Figure. 1B). Furthermore, transfer of nucleotide sequences between viral particles in the supplemented of the myeloma cells cultural fluid and normal mouse embryonic fibroblasts was proposed on the influence of cryo-protector DMSO and drastic temperature changes. After freezing of the so prepared mixed cultures in the presence of DMSO, subsequent thawing and re-incubation, appearance of hybrid cells of myeloid-like and phagocyte-like cells, as well as lymphoid-like and plasmatic cells with malignant myeloma cells, were noted (Figure. 1C). On the same principle, possibilities about production of cellular and recombinant viral vaccines by exchange of nucleotide sequences between viral particles and cells were proposed (Figure. 1B).

Discussion

One of the explanations of the noted changes could be the proved in the literature existence of able to differentiate in various directions stem-like cellular sub-populations in the general 3T3 cell line [7,10]. The results obtained suggested the role of DMSO as an activator of fusion processes, which was probably activated also by the drastic temperature changes, and were in confirmation agreement with literature data for this substance probably due to changes in the membranes structures and properties. Furthermore, the data might propose transfer of nucleotide (DNA- and/or RNA-) fragments with anti-malignant activity between cells and viral particles. In this way, here again was proposed exchange of nucleotide sequences between separate cells, but also between cells and viral particles, and thus, derivation of cells and nucleotide sequences with immunogenic/vaccine and anti-viral properties, including by RNA-interference of genes, coding malignant or viral proteins. In this way, a possibility about production of membrane receptor glycoproteins by non-myeloid and non-lymphoid cells in appropriate conditions (as for instance, availability of viral antigens, with and/or without the presence of immunomodulators) was suggested. These results suggested a possibility about derivation of any type of cells by application of appropriate incubation techniques. Also, the presented results showed a possibility for production of membrane glycoprotein receptors on the surface of non-lymphoid and non-myeloid cellular types in appropriate conditions as viral antigens, as well as of different types of immunomodulators.

The application of cytokines, as well as diversified prime and boost, combined with viral infiltration, has been determined as key factors for stimulation of T-lymphocyte response. On the other hand, appearance of "intrinsic antiviral immunity" changes as internal protection of the cell in response to its infection has been proved [11,12]. In this way, the usability of this technique for fusion procedures as between separated cells and of cells with viral particles, for gene-engineering and biotechnological needs. Myeloid and lymphoid cells have shown capabilities for differentiation in respective directions in transfer in them of appropriate nucleotides, coding respective antigens, on one hand, but also as usable novel safe vaccines, carrying genes for respective malignant antigens or cytokines, on the other [5].

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