



# Development of Techniques about Production of Cells and Nucleotide Sequences with Immunogenic Properties

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## Abstract

*In vitro*-cultures of normal mouse embryonic fibroblasts from 3T3 cell line, but also of mouse malignant myeloma cells, as well as mixed cultures of both cellular types were prepared and proceeded in different conditions. In the mouse malignant myeloma cells, containing inserted Murine Leukemia Virus (MuLV) (with RNA-genome, *Retroviridae* family), signs of increased malignant *in vitro*-potential were noted in comparison to non-containing mouse myeloma cells as decreased sizes and number of the myeloma cells, but also their decreased capability for 3D-growth. In pre-incubation of normal mouse embryonic fibroblasts in cultural fluid, supplemented by previous cultivation of mouse malignant myeloma cells in it, followed by freezing in the presence of cryo-protector Dimethylsulfoxide (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. In co-cultivation of normal mouse embryonic fibroblasts with mouse malignant myeloma cells, containing inserted MuLV, followed by freezing in the presence of DMSO, subsequent thawing and re-incubation, exchange of nucleotide sequences between viral particles and normal mouse cells was proposed on the influence of the used organic detergent, as well as of the drastic temperature changes. Possibilities about production of cellular and recombinant viral vaccines by exchange of nucleotide sequences between viral particles and cells could be suggested.

**Keywords:** Myeloid and Lymphoid Differentiation; Activated Fusion; Exchange of Nucleotide Sequences

## Introduction

The techniques for *in vitro*-cultivation of viruses in cell cultures allow the design of various viral recombinants, including of recombinant viral vaccines. As appropriate tools can be used for DNA- and RNA-viruses [1,2-5], but also bacteria plasmids and yeasts [4,6]. For this aim, an intact *tk* gene, coding the enzyme thymidine kinase (TK), has been found to be necessary, but, on the other hand – the integration of the searched gene(s) out of *tk* locus of the viral genome, as well as virus promoter, which could provide the expression of the inserted gene(s). Changes in the promoter and/or 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

have been inserted genes, coding cell receptors, cytokines, enzymes, complement activators, apoptosis activators and/or inhibitors, surface antigens, tumor markers. Besides the respective inserted gene(s), a marker gene has also been found to be necessary, but both gene types are controlled by appropriate promoter sequences. Most often, as marker genes could be used such, which code color proteins (for example, fluorescent protein, as GFP, YFP, RFP) or products, participating in a color reaction (enzymes luciferase or thymidine kinase), or is connected in any way resistance to any antibiotic preparation [3]. 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 endonucleases). Other important components are the reverted end DNA-repeats. In this connection, the main goal of the current study is directed to development of *in vitro*-methods of protection and immunogenicity.

### 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) 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°C in incubator with 5% CO<sub>2</sub> 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 frozen in the presence of cryo-protector Dimethylsulfoxide (DMSO), subsequently thawed and re-cultivated in fresh medium in the standard conditions, described above.

### Results

In the mouse malignant myeloma cells, containing inserted MuLV, were noted signs of increased malignant *in vitro*-potential (Figure 1A), compared to non-containing mouse myeloma cells (Figure 1B). As signs were accepted the observed decreased sizes and number of the myeloma cells, but also their decreased capability for 3D-growth (Figure 1B). In pre-incubation of normal mouse embryonic fibroblasts in cultural fluid, supplemented by previous cultivation of mouse malignant myeloma cells in it, subsequent freezing in the presence of cryo-protector DMSO, followed by 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). In co-cultivation of normal mouse embryonic fibroblasts with mouse malignant myeloma cells, containing

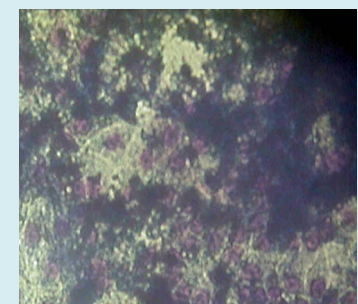
inserted MuLV, subsequent freezing in the presence of DMSO, followed by thawing and re-incubation, exchange of nucleotide sequences between viral particles and normal mouse cells was proposed on the influence of the used organic detergent, as well as of the drastic temperature changes (Figure 1D). On this principle, possibilities about production of cellular and recombinant viral vaccines by exchange of nucleotide sequences between viral particles and cells could be suggested.



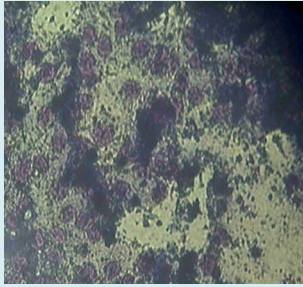
**Figure 1A:** mouse malignant myeloma cells, non-containing inserted viral genome sequences.



**Figure 1B:** mouse malignant myeloma cells, transfected previously with MuLV.



**Figure 1C:** incubated in cultural fluid from mouse malignant myeloma cells (eventually containing nucleotide sequences from the transforming vector), frozen 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.



**Figure 1D:** co-cultivated with mouse malignant myeloma cells, frozen 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.

## Discussion

The results obtained could be explained with activated fusion between separate cells on the influence of DMSO, as well as of the drastic temperature changes. They were in confirmation of literature data about the activated fusion on the influence of DMSO. The observed changes could be explained with eventual existence of sub-populations of stem-like cells in the general normal embryonic 3T3 cell line, which are able to differentiate in various directions, depending of the respective incubation conditions. Messages about similarly derived hybrid cells have been received in fusion of malignant cells with myeloid and lymphoid cells [7], but also with normal fibroblasts [8]. The current data also suggested a possibility for appearance of membrane glycoprotein receptors on the surface cells in initial phases of differentiation in appropriate conditions as for instance viral and/or malignant antigens, as well as different types of appropriate immunomodulators. These results were in agreement with the literature findings in this direction [9,10-13]. On the other hand, both 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. Furthermore, the existence of “intrinsic antiviral immunity” changes, as internal protection of the cell in response to its infection has been proved [14,15]. Another explanation could be connected with the proved action of some cell-produced proteins as glycoprotein receptors to specific bio-molecules [16]. In this way, the current data supported the importance of various intra- and extra-cellular inter-molecular interactions in cascade regulatory pathways, underlining functions of the

different types of cells [17,18].

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