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Full Length Research Paper

Differentiation of stem and progenitor cells in activated immune cells with anti-malignant properties

Iskra Ventseslavova Sainova*, Ilina Vavrek, Velichka Pavlova, Ivan Iliev, Lilija Yossifova, Elena Gardeva and Elena Nikolova

Department of Experimental Morphology, Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, "Acad. G. Bonchev" Str., 1113 Sofia, Bulgaria.

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Studies on the biology of immune cells are mainly focused on their role as immune activators and modulators. In their appropriate cultivation and/or modifications, they have shown abilities for an enhanced expression of specific effective molecules. These properties have characterized them as promising candidates for construction of novel safe vaccines and gene-engineering products on their basis. In this aspect, in the last years, attention is directed towards the development of new safe therapeutic methods and techniques with immune cells. In this connection, according to results obtained, *in vitro*-co-cultivation derived by myeloid and lymphoid precursors with positive and negative on the additionally-inserted copy of the oncogene *Dcn1* transfected cells, no immune reaction is observed against both genetically-manipulated cell types, which could be accepted as a proof for their safety. On the other hand, signs of increased degree for both myeloid and lymphoid differentiation in the presence of transfected cells, positive on additionally-inserted copy of the oncogen are indicated.

Key words: Progenitor cells, immune cell precursors, gene constructs, oncogenes, tumor-suppresser genes.

INTRODUCTION

In the last years, the development of novel therapeutic strategies with immune cells has become extensively investigated (Chaussabel and Banchereau, 2005; Crispin and Alcocer-Varela, 2007). Possibilities for appropriate modifications of immune cells for expression of malignancy-specific antigens of them by *in vitro* and/or *ex vivo*-transfer of genes, coding respective antibodies, have also been suggested (Crispin and Alcocer-Varela, 2007; Yongqing et al., 2002). So, genetically modified dendritic cells (DCs) have been widely tested in pre-clinical studies, including anti-malignant agents. After such application of DCs, peptide-specific responses by cytotoxic T-lymphocytes (CTLs), improvement in performance status, decrease in malignancy markers levels, regression of malignancies and at the same time, no toxic side effects have been accounted. In the light of the unique properties of immune cells, they have also been proposed as powerful immunomodulation agents, included in the composition of novel vaccines and gene-engineering products for treatment of malignant disorders (Crispin and Alcocer-Varela, 2007; Yongqing et al., 2002; Aicher et al., 1997). By use of polymerase chain reaction (PCR) in real time-PCR (RT-PCR), ability for initiation of erythroid (β-globin) and/or myeloid (myeloperoxidase) gene expression programs by the same cell prior to exclusive commitment to the erythroid, respectively, myeloid lineages for it, has been shown. Furthermore, according to many literature data, granulocyte-macrophage colony-stimulating factor (GM-CSF) mobilizes CD34+ bone-marrow progenitor cells both in vitro and in vivo with an increased frequency and generation of DCs with anti-malignant properties (Gong et al., 1997). Similarly, the addition of GM-CSF plus tumor necrosis factor-α (TNF-α), has been found to induce development of DCs from purified CD34+ cells of bone marrow, cord blood and peripheral blood. Increased Th1 cytokine production and stronger anti-malignant effect have not been observed in mice, depleted of gamma-interferon (IFN-γ), which has also supported the maintenance of "immune cells-malignant cells" conjugates as potent anti-malignant vaccines (Xia et al., 2004). These data could be additionally administrated by gene transfection of cells

*Corresponding author. E-mail: isainova@yahoo.com.

for enhancement of the immunity, probably by IFN-γ mediation. For further increase of the potency of the vaccine, a combined variation of both technologies has been applied, in which IL-18-transfected immune cells have been used. It has also been indicated that GM-CSF gene-modified DCs might lead to generation of hybrid vaccines with potentially increased therapeutic efficacy (Brown et al., 2000). Similarly, results from experiments for immunization with fusion hybrids, derived by fusion of immune cells with IL-12 gene-transferred malignant cells, have shown an ability to elicit a previously enhanced anti-malignant effect in experimental therapeutic models (Xia et al., 2004). Such novel IL-12-producing fusion cell vaccine has been characterized as a promising inter-vention for future immune therapy of malignant diseases.

Undifferentiated tissue stem cells could circulate in the body and contribute to repair if needed (Kashofer and Bonnet, 2005). In this connection, different types of immune cells have been found to play a pivotal role in the processes of immune response initiation and modulation (Gonzalez-Rey et al., 2006; Perona-Wright et al., 2007). On the other hand, they have been found to participate in the maintenance of peripheral tolerance, and their capacity to induce anti-nuclear auto-immune response has been proven in experimental models (Summers-Deluca et al., 2007). The main goal was connected with providing of safe way about active oncogenes over-expression for eventual prevention of early aging and death *in vitro*.

MATERIALS AND METHODS

Stem cells isolated from mouse Balb/c embryos were cultivated for 48 to 72 h on previously formed monolayers of feeder primary mouse embryonic fibroblasts (MEFs) after their previous treatment by Mitomycin-c (mm-c) (Sigma-Aldrich) and/or 3T3 fibroblasts. After tripsinization, they were transfected by electroporation (5×10^6 cells/ml). For this aim, recombinant DNA-genome from adeno-associated virus (AAV) (*Parvoviridae*) (Chen et al., 2003) containing promoter for gene, coding elongation factor 1-alpha (*EF1-α*), gene *Dcn1*, isolated from 3T3 fibroblasts of laboratory mice Balb/c, as well as gene for neomycin resistance and isolated from bacterial DNA-plasmid are used. For this goal, electroporator for cell transfection (BioRad) was used. On the other hand, cell cultures derived from human cervical carcinoma cell line Hela were also prepared. All cells were incubated at 37°C in incubator with 5% CO_2 and 95% air humidification, in 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), and were observed by inverted light microscope (Leica).

After tripsinization of the transfected cells and their consequent treatment with mixture of phenol-chlorophorm-isoamil alcohol (PCI) (Sigma-Aldrich), the isolated nuclear material was treated with lysis buffer (Sigma-Aldrich) for isolation of genomic DNA. The last was subjected on polymerase chain reaction (PCR) of previously isolated nuclear DNA of them and its consequent 1% agarose gel (Sigma-Aldrich) electrophorhesis, in the presence of DNA-primers against the inserted DNA-fragment (Sigma-Aldrich), mixture of the four types desoxy-nucleosid-tri-phosphates (dNTP - Sigma-Aldrich) and enzyme Taq-polymerase (Sigma-Aldrich).

For differentiation in myeloid and lymphoid precursors, populations of non-transfected mESCs were also cultivated in medium containing GM-CSF (Sigma-Aldrich) and complement proteins, respectively, by addition of 10% non-inactivated FCS (Sigma-Aldrich) in the last case. Consequently, to the cell sub-populations of both populations of non-transfected cells were added malignant antigens. The last were derived by cultivation of Hela cells in serum-free DMEM (Sigma-Aldrich) for 24 h, its consequent centrifugation and filtration. Fixed light microscopic preparations were prepared by their consequent fixation by treatment with 95% ethanol (Sigma-Aldrich) or paraphormaldehyde (Sigma-Aldrich), washing with 1:9 diluted PBS (Sigma-Aldrich) and Giemsa-staining (Sigma-Aldrich).

RESULTS AND DISCUSSION

In our experiments, 9 transfected electroporation cell clones were received and derived. According to the genomic assays results, only 2 of them were positive on the additionally inserted copy of the oncogene *Dcn1* and the other 7 cell clones-negative on it (Figure 1). Signs of early myeloid cell differentiation were noticed in *in vitro*-co-cultivation of non-transfected ESCs populations in the presence of GM-CSF and malignant antigens (Figure 2d), but not in the presence of only GM-CSF (Figure 2b) or only malignant antigens (Figure 2c). Signs of further myeloid differentiation were observed in laboratory co-cultivation with transfected cells, positive on additional-inserted copy of the oncogene *Dcn1*, but not in the presence of transfected cells, negative on it.

Similarly, in laboratory cultivation of non-transfected mESCs in the presence of complement components and malignant cell antigens, signs of early lymphoid cell differentiation were noticed (Figure 3d). These changes were not observed in the presence of only complement proteins (Figure 3b) or only malignant antigens (Figure 3c). In their *in vitro*-co-cultivation with transfected cells, positive on additional copy of the oncogene *Dcn1*, signs of further lymphoid differentiation were also noticed (Figure 3f), but not in the presence of transfected cells, negative on it (Figure 3e).

On the other hand, in both cases, no signs of malignant *in vitro*-transformation of both types of transfected cells were observed (Figures 2 and 3).

Rapid lymphoid-restricted (T-, B- and NK) reconstitution capacity *in vivo*, but completely lacked myeloid differentiation potential both *in vivo* and *in vitro*, has been reported in stem cells from bone marrow material of adult laboratory mice (Kobari et al., 2000). On the other hand, sequential stimulation with immune cells-breast carcinoma cells fusion hybrids, which has resulted in a marked expansion of activated malignancy-specific T-lymphocytes has been demonstrated in recently published literature data.

Conclusion

Cells treated with both GM-CSF and malignant antigens,

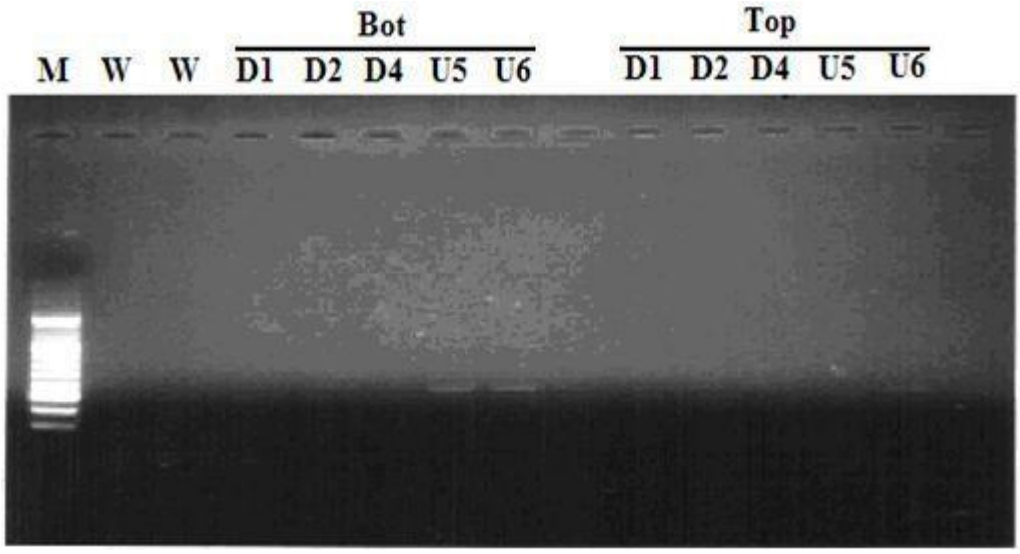

Figure 1. 1% agarose gel electrophorhesis for proof of the presence and/or the absence of oncogene *Dcn1*, by use of DNA-primers against the recombinant gene construct for proof of the presence and/or the absence of additionally-inserted copy of the oncogene *Dcn1* in clones, derived from transfected by electroporation *in vitro*-cultivated mESCs.

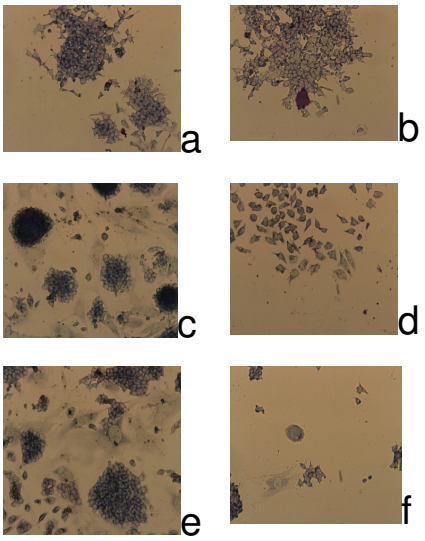

Figure 2. Early stages of myeloid *in vitro*-differentiation of non-transfected mESCs in different conditions: in the absence of differentiation factors and transfected cells (a); in the presence of GM-CSF, but in the absence of Hela-antigens and transfected cells (b); in the presence of Hela-antigens and in the absence of GM-CSF and transfected cells (c); in the presence of both GM-CSF and Hela-antigens, but in the absence of transfected cells (d); in the presence of GM-CSF, Hela-antigens and transfected cells, negative by additionally-inserted copy of oncogene *Dcn1* (e); in the presence of GM-CSF, Hela-antigens and transfected cells, positive on additionally-inserted copy of oncogene *Dcn1* (f).

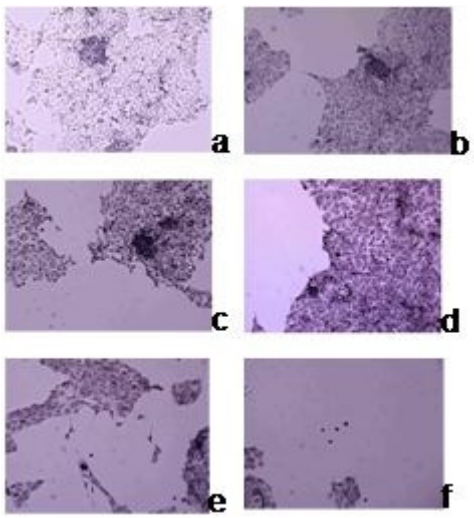

Figure 3. Early stages of lymphoid *in vitro*-differentiation of mESCs indifferent conditions: in the absence of differentiation factors and transfected cells (a); in the presence of complement components and absence of Hela-antigens and transfected cells (b); in the presence of Hela-antigens, but in the absence of complement proteins and transfected cells (c); in the presence of both complement components and Hela-antigens, but in the absence of transfected cells (d); in the presence of complement proteins, Hela-antigens and transfected cells, negative by additionally-inserted copy of oncogene *Dcn1* (e); in the presence of complement proteins, Hela-antigens and transfected cells, positive on additionally-inserted copy of oncogene *Dcn1* (f).

have indicated signs of early myeloid differentiation. Similarly, in the presence of both complement components and malignant antigens, signs of early differentiation in lymphoid cell precursors were noticed. These changes were not observed in control non-transfected ESCs, as well as in that cultivated in the presence of only GM-CSF, complement proteins and malignant antigens, which have shown reserved stem-cell characteristics. In both cases, signs of further myeloid and lymphoid cell differentiation were observed in the presence of derived transfected cells, positive on additionally-inserted copy of the oncogene *Dcn1*, but not in the presence of obtained transfected cells, negative on it. On the other hand, no signs of malignant transformations of all received transfected cells were noticed. These data could also be accepted as a proof for the safety of the derived transfected cell types.

REFERENCES

Aicher A, Westermann J, Cayeur S, Willimsky G, Daemen K, Blankestein T, Uckert W, Dörken B, Pezzutto A (1997). Successful *retroviral* mediated transduction of a reporter gene in human dendritic cells: feasibility of therapy with gene-modified antigen presenting cells. Exp. Hematol., 25: 3-44.

Brown M, Zhang Y, Dermine S, De Wynter EA, Hart C, Kitchener H, Stern PL, Skinner MA, Stacey SN (2000). Dendritic cells infected with recombinant *fowlpox virus* vectors are potent and long-acting stimulators of transgene-specific class I restricted T-lymphocyte activity. Gene Ther., 7: 1680–1689.

Chaussabel D, Banchereau J (2005). Expression of lymphotoxin-alphabeta on antigen-specific T cells is required for DC function. Dendritic cells, therapeutic vectors of immunity and tolerance. Am. J. Transpl., 5: 205–206.

Chen S, Agarwal A, Glushakova OY, Jorgensen MS, Salgar SK, Poirier A, Flotte TR, Croker BP, Madsen KM, Atkinson MA, Hauswirth WW, Berns KI, Tisher CC (2003). Gene delivery in renal tubular epithelial cells using recombinant adeno associated viral vectors. J. Am. Soc. Nephrol., 14: 947-958.

Crispin JC, Alcocer-Varela J (2007). The role myeloid dendritic cells play in the pathogenesis of systemic lupus erythematosus. Autoimmun. Rev., 6: 450–456.

Gong J, Chen D, Kashiwaba M, Kufe D (1997). Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. Nat. Med., 3: 558–561.

Gonzalez-Rey E, Chorny A, Fernandez-Martin A, Ganea D, Delgado M (2006). Vasoactive intestinal peptide induces regulatory dendritic cells that can prevent acute graft-versus-host disease while maintain graft-versus-tumor response. Blood, 107: 3632–3638.

Kashofer K, Bonnet D (2005). Gene therapy progress and prospects: stem cell plasticity. Gene Ther., 12: 1229–1234.

Kobari L, Pflumio F, Giarratana M, Li X, Titeux M, Izac B, Leteuirtre F, Coulombel L, Douay L (2000). *In vitro*- and *in vivo*-evidence for the long-term multilineage (myeloid, B-, NK, and T-) reconstitution capacity of *ex vivo*-expanded human CD34(+) cord blood cells. Exp. Hematol., 28: 1470-1480.

Perona-Wright G, Anderton SM, Howie SEM, Gray D (2007). IL-10 permits transient activation of dendritic cells to tolerize T-cells and protect from central nervous system autoimmune disease. Int. Immunol., 19: 1123–1134.

Summers-Deluca LE, McCarthy DD, Cosovic B, Ward LA, Lo CC, Scheu S, Pfeffer K, Gommerman JL (2007). J. Exp. Med., 204: 1071–1081.

Xia D, Li F, Xiang J (2004). Engineered fusion hybrid vaccine of *IL-18* gene-modified tumor cells and dendritic cells induces enhanced antitumor immunity. Cancer Biother. Radiopharm., 19: 322–330.

Yongqing Z, Weindong T, Chan WT, Saxena A, Xiang J (2002). Engineered fusion hybrid vaccine of IL-4 gene-modified myeloma and relative mature dendritic cells enhances antitumor immunity. Leukemia Res., 26: 757–763.