FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 43, No. 4, 2005
pp. 213-216

Review article

Messenger RNA electroporation: an efficient tool in immunotherapy and stem cell research

A. Van Driessche, P. Ponsaerts, D.R. Van Bockstaele, V.F.I. Van Tendeloo and Z.N. Berneman

Laboratory of Experimental Hematology, Faculty of Medicine, University of Antwerp, Belgium

Abstract: Over the last decades medicine has developed tremendously, but still many diseases are incurable. The last years, cellular (gene) therapy has become a hot topic in biomedical research for the potential treatment of cancer, AIDS and diseases involving cell loss or degeneration. Here, we will focus on two major areas within cellular therapy, cellular immunotherapy and stem cell therapy, that could benefit from the introduction of neo-expressed genes through mRNA electroporation for basic research as well as for clinical applications. For cellular immunotherapy, we will provide a state-of-the-art on loading antigen-presenting cells with antigens in the mRNA format for manipulation of T cell immunity. In the area of stem cell research, we will highlight current gene transfer methods into adult and embryonic stem cells and discuss the use of mRNA electroporation for controlling guided differentiation of stem cells into specialized cell lineages.

Key words: mRNA - Electroporation - Immunotherapy - Stem cells - Antigen presenting cells - Dendritic cells

Introduction

The knowledge of medicine, immunology and biotechnology has progressed enormously, but still many diseases are incurable and have a large impact on the quality of life. Therefore many scientists are looking for new treatments for several diseases, like cancer, auto-immune diseases, pandemic infections (*e.g.* HIV), neurodegenerative diseases, *etc*. Several different approaches are studied, *e.g.* new sorts of drugs and combinations of radiotherapy and chemotherapy, but during the last years much attention has been paid to custom-made cellular therapies. In this review we will focus on the application of cellular therapy and mRNA electroporation in different cell types for the potential treatment of various diseases.

Use of mRNA electroporation for immunotherapy

The concept of immunotherapy in cancer and infectious diseases is based on the body's natural defence system that protects us against a variety of diseases and is a therapy that attempts to modify or enhance immune responses. The most studied cell types for immunotherapy are antigen-presenting cells (APC) and T cells, which are pivotal players in initiating immune responses. Different strategies have been developed for loading APC with tumor or viral antigens [37]. Loading of characterized antigenic peptides is the most straightforward way, however disadvantages are the prior knowledge of the peptide epitopes, the short half-life of the human leukocyte antigen (HLA)/peptide complexes and the dependence on screening for appropriate HLA haplotypes in individual patients. Alternatively, viral and non-viral gene transfer technologies can be used. Recombinant viral vectors are characterized by a high transduction efficiency, but pose biosafety risks for clinical application; while transduction efficiency with viral vectors is high [10], plasmid DNA transfer into dendritic cells (DC), despite high clinical potential, has not been very efficient [34]. Nonviral non-DNA based gene delivery has several advantages, as compared to plasmid DNA and viral vectors, since there is no danger of insertional mutagenesis, no viral antigens and absence of vector-induced immunogenicity. The first report on the use of mRNA to load APC has come from the group of E. Gilboa [2,4,18]. The group of Gilboa applied passive RNA pulsing or RNA lipofection to introduce the coding RNA

Correspondence: Z.N. Berneman, Division of Hematology, Antwerp University Hospital, 10 Wilrijkstraat, B-2650 Edegem, Belgium; e-mail: zwi.berneman@uza.be

Lecture presented at the Third Annual Meeting of the European Stem Cell Therapeutics Excellence Centre, October 6-9, 2005, Cracow, Poland

into DC. Our group was the first to describe an optimized electroporation protocol for the introduction of RNA into DC providing both biochemical evidence of transfection in terms of transgene EGFP expression as well as evidence of major histocompatibility complex (MHC) class I presentation [33]. Moreover, we demonstrated that mRNA electroporation of human DC was more efficient than plasmid DNA electroporation, and lipofection or passive pulsing of RNA for stimulation of antigen-specific CD8+ T cells, as confirmed by several other groups [11, 15, 27].

Dendritic cells

Thus far, most reports on mRNA electroporation have used dendritic cells (DC). As 'professional' antigen-capturing cells in an immature state and antigen-presenting cells after maturation, they play an important role in the activation of innate and adaptive immunity to pathogens, as well as in the maintenance of peripheral tolerance [30]. Especially the expression of costimulatory molecules on DC provides an extra immune stimulating signal to initiate a more efficient immune response. DC can be cultured from peripheral blood monocytes and therefore are readily accessible for use in cellular therapy. Antigen processing and subsequent MHC class I presentation on DC membrane after mRNA electroporation could be demonstrated using a CD8+ T cell clone for MAGE-A1 [24], Melan A [33] and WT1 (unpublished results). Transfection of mRNA encoding influenza (flu) matrix protein M1 allows loading of monocytes or DC for induction of autologous flu-specific CD8+ T cell activation [20, 22, 25]. After cryopreservation, mRNA-electroporated DC retained transgene expression, phenotypic properties and, most importantly, stimulatory capacity [25]. Recently, more reports on *in vitro* activation using RNA-electroporated dendritic cells have been published, generally regarding cancer-related antigens, *e.g.* telomerase [27], Melan A [11], total myeloma cell RNA [17]. Recently, we have observed that electroporation of HIV-1 *gag* mRNA can activate memory T cells from HIV patients *ex vivo* (unpublished results). Patients' autologous proviral DNA was PCR-amplified and DC electroporated with *in vitro* transcribed proviral *gag* mRNA stimulated autologous T cells. These findings open a major perspective for the development of patient-specific immunotherapy directed against the entire latent HIV quasispecies.

The loading of cells with mRNA is not only useful to transfect antigens, but can also be used to introduce costimulatory molecules in dendritic cells, like toll-like receptor (TLR) 4 [1, 5] and OX40L [7].

The use of mRNA-loaded DC has been very successful in *in vitro* experiments and now this strategy has been translated into several clinical trials. Cytotoxic T lymphocyte (CTL) responses were obtained in patients with metastatic prostate tumors after vaccination with DC passively pulsed with prostate-specific antigen mRNA [9]. Nair *et al*. showed that vaccination with DC transfected with total tumor-derived mRNA stimulated a tumor-specific immune response in a patient with a carcino-embryonic antigen (CEA)-expressing adenocarcinoma [19]. Other clinical trials using RNA-loaded DC are still ongoing. We are currently recruiting acute myeloid leukemia (AML) patients in remission for a vaccine trial using WT1 mRNA-electroporated DC.

CD40-activated B cells

B cells are an alternative for DC and can be obtained from small quantities of peripheral blood. In contrast to the culture of DC starting from monocytes, B cells can proliferate and massive amounts can be cultured *in vitro* by CD40 activation, which makes them a cost-effective alternative for DC [28]. CD40-activated (CD40-) B cells have been shown in several *in vitro* systems to activate CTL responses against viral and tumor antigen targets, including neoantigens, that are formulated as peptides, proteins, or viral vectors [3, 12, 14]. Coughlin *et al*. reported that CD40-B cells transfected with RNA may serve as an alternative vaccine that can be generated from small blood volumes regardless of patient age [6]. They cultured CD40-B cells from pediatric patients with neuroblastoma and elicited cytotoxic T cells against several neuroblastoma-related antigens using tumor RNA-electroporated CD40-B cells. CD40-B cells can also be used to elicit CTL responses against infectious agents as demonstrated by Van den Bosch *et al*. for cytomegalovirus and influenza [31].

T cells

In the field of adoptive T cell immunotherapy, mRNA-based gene transfer into T cells could prove useful. Our group demonstrated that mRNA electroporation is feasible in activated T cells. However, in unstimulated T cells, mRNA electroporation turned out to be relatively inefficient [29].

Use of mRNA electroporation in stem cell research

Stem cell research, both embryonic and adult, offers several prospects towards the development of future cell-based therapies in regenerative medicine. The hope exists that (stem) cell transplantation will become part of an effective therapy for neurological disease or injury, diabetes and myocardial infarction. Although various combinations of growth factors and chemicals have been described for *in vitro* differentiation of stem cells into specific cell types, novel research is now focusing on the development of gene-based strategies in order to control and/or direct *in vivo* differentiation of transplanted stem

cell populations. For this, viral transduction techniques have been described as highly efficient for genetic modification of adult and embryonic stem cell populations [8, 16, 35], while currently optimized non-viral plasmid DNA-based transfection methods do not result in exceptionally high gene transfer efficiencies [13, 36]. As described before, one should consider the use of non-viral gene transfer methods when looking forward to clinical applications. In this context, we have investigated and introduced potential applications of mRNA-based gene transfer in stem cell research.

RNA-based gene transfer for adult and embryonic stem cells

For adult stem cell populations, we have shown that mRNA electroporation is a highly efficient gene transfer technology for *in vitro* cultured human bone marrow stromal cells (90% of gene transfer efficiency) and can also be used, although less efficiently at this time, for genetic loading of human CD34+ hematopoietic stem cells (35% of gene transfer efficiency) [29]. For embryonic stem cell populations, we demonstrated highly efficient cytoplasmic gene expression in 80-90% of mouse and human embryonic carcinoma (EC) stem cells (unpublished data) and mouse and human embryonic stem (ES) cells after electroporation with *in vitro* transcribed mRNA [21, 23, 26]. Moreover, next to the possibility of short term gene transfer via direct mRNA electroporation, electroporation with mRNA encoding Cre- or FLPe-recombinase proteins provides an easy and highly efficient method to induce sustained transgene expression in whole ES cell populations stably transfected with LoxP and/or FRT-flanked target sequences [21, 32].

Directing stem cell differentiation via RNA-based gene transfer

One of the research lines in our laboratory aims to investigate whether directed neural differentiation of embryonic cells can be induced after electroporation with mRNA encoding neural transcription factors and/or growth factors. In preliminary experiments, mouse P19 EC stem cells were electroporated with mRNA encoding various neurotrophic factors. Next, mRNA-electroporated P19 EC stem cells were allowed to form embryoid bodies during 4 days followed by flow cytometric analysis for the presence of neural progenitor cells based on A2B5 staining. While mock electroporation or electroporation with mRNA encoding EGFP, brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) did not result in directed neural differentiation, electroporation with mRNA encoding neurotrophin-3 (NT3) or electroporation with a mixture of BDNF, GDNF and NT3 mRNA resulted in an increased number of A2B5+ neural progenitor cells within cultured embryoid bodies (unpublished data). These encouraging results on P19 EC stem cells provide proof-of-principle for the further development of RNA-based gene transfer strategies for triggering stem cell differentiation.

Conclusions and future perspectives

As outlined above, mRNA electroporation is a versatile powerful tool for obtaining short-term transgene expression in a variety of cell types. The use of mRNA-loaded DC has been very successful for *in vitro* purposes and has been recently translated into several clinical trials. The first results are very encouraging, as tumor specific immune responses were elicited [9, 19]. Several other clinical trials using RNA-loaded DC are still ongoing and will hopefully pave the way for improved DC therapy of cancer, but also for infectious diseases.

Messenger RNA electroporation is a promising method for genetic loading of stem cells with genes in order to direct their differentiation towards specialized cell types, which could be of use in the treatment of various diseases. However, clinical applicability can only be established after intensive laboratory studies, both *in vitro* and *in vivo*, that should elaborate on the safety and effectiveness of stem cell-based therapies.

Acknowledgements: This work was supported by grant n G.0456.03, G.0313.01, 7.0004.03N and WO.012.02 of the Fund for Scientific Research - Flanders, Belgium (FWO-Vlaanderen), by grants of the Fortis Bank Verzekeringen-financed Cancer Research, by a research grant of the Belgian Federation against Cancer (BFK) and by the Fund for Cell Therapy from the Antwerp University Hospital (UZA). AVD holds a PhD fellowship of the Emmanuel van der Schueren Foundation. V.F.I.V.T. is a postdoctoral fellow of the Fund for Scientific Research - Flanders, Belgium (FWO-Vlaanderen).

References

[1] Abdel-Wahab Z, Cisco R, Dannull J, Ueno T, Abdel-Wahab O, Kalady MF, Onaitis MW, Tyler DS, Pruitt SK (2005) Cotransfection of DC with TLR4 and MART-1 RNA induces MART-1-specific responses. J Surg Res 124: 264-273

[2] Ashley DM, Faiola B, Nair S, Hale LP, Bigner DD, Gilboa E (1997) Bone marrow-generated dendritic cells pulsed with tumor extracts or tumor RNA induce antitumor immunity against central nervous system tumors. J Exp Med 186: 1177-1182

[3] Bergwelt-Baildon MS, Vonderheide RH, Maecker B, Hirano N, Anderson KS, Butler MO, Xia Z, Zeng WY, Wucherpfennig KW, Nadler LM, Schultze JL (2002) Human primary and memory cytotoxic T lymphocyte responses are efficiently induced by means of CD40-activated B cells as antigen-presenting cells: potential for clinical application. Blood 99: 3319-3325

[4] Boczkowski D, Nair SK, Snyder D, Gilboa E (1996) Dendritic cells pulsed with RNA are potent antigen-presenting cells i*n vitro* and *in vivo*. J Exp Med 184: 465-472

[5] Cisco RM, Abdel-Wahab Z, Dannull J, Nair S, Tyler DS, Gilboa E, Vieweg J, Daaka Y, Pruitt SK (2004) Induction of human dendritic cell maturation using transfection with RNA encoding a dominant positive toll-like receptor 4. J Immunol 172: 7162-7168

[6] Coughlin CM, Vance BA, Grupp SA, Vonderheide RH (2004) RNA-transfected CD40-activated B cells induce functional T-

cell responses against viral and tumor antigen targets: implications for pediatric immunotherapy. Blood 103: 2046-2054
[7] Dannull J, Nair S, Su Z, Boczkowski D, Debeck C, Yang B, Gilboa E, Vieweg J (2005) Enhancing the immunostimulatory function of dendritic cells by transfection with mRNA encoding OX40 ligand. Blood 105: 3206-3213
[8] Gropp M, Itsykson P, Singer O, Ben Hur T, Reinhartz E, Galun E, Reubinoff BE (2003) Stable genetic modification of human embryonic stem cells by lentiviral vectors. Mol Ther 7: 281-287
[9] Heiser A, Coleman D, Dannull J, Yancey D, Maurice MA, Lallas CD, Dahm P, Niedzwiecki D, Gilboa E, Vieweg J (2002) Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors. J Clin Invest 109: 409-417
[10] Jenne L, Schuler G, Steinkasserer A (2001) Viral vectors for dendritic cell-based immunotherapy. Trends Immunol 22: 102-107
[11] Kalady MF, Onaitis MW, Padilla KM, Emani S, Tyler DS, Pruitt SK (2002) Enhanced dendritic cell antigen presentation in RNA-based immunotherapy. J Surg Res 105: 17-24
[12] Kondo E, Topp MS, Kiem HP, Obata Y, Morishima Y, Kuzushima K, Tanimoto M, Harada M, Takahashi T, Akatsuka Y (2002) Efficient generation of antigen-specific cytotoxic T cells using retrovirally transduced CD40-activated B cells. J Immunol 169: 2164-2171
[13] Lakshmipathy U, Pelacho B, Sudo K, Linehan JL, Coucouvanis E, Kaufman DS, Verfaillie CM (2004) Efficient transfection of embryonic and adult stem cells. Stem Cells 22: 531-543
[14] Lapointe R, Bellemare-Pelletier A, Housseau F, Thibodeau J, Hwu P (2003) CD40-stimulated B lymphocytes pulsed with tumor antigens are effective antigen-presenting cells that can generate specific T cells. Cancer Res 63: 2836-2843
[15] Lundqvist A, Noffz G, Pavlenko M, Saeboe-Larssen S, Fong T, Maitland N, Pisa P (2002) Nonviral and viral gene transfer into different subsets of human dendritic cells yield comparable efficiency of transfection. J Immunother 25: 445-454
[16] Ma Y, Ramezani A, Lewis R, Hawley RG, Thomson JA (2003) High-level sustained transgene expression in human embryonic stem cells using lentiviral vectors. Stem Cells 21: 111-117
[17] Milazzo C, Reichardt VL, Muller MR, Grunebach F, Brossart P (2003) Induction of myeloma-specific cytotoxic T cells using dendritic cells transfected with tumor-derived RNA. Blood 101: 977-982
[18] Nair SK, Boczkowski D, Morse M, Cumming RI, Lyerly HK, Gilboa E (1998) Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes *in vitro* using human dendritic cells transfected with RNA. Nat Biotechnol 16: 364-369
[19] Nair SK, Morse M, Boczkowski D, Cumming RI, Vasovic L, Gilboa E, Lyerly HK (2002) Induction of tumor-specific cytotoxic T lymphocytes in cancer patients by autologous tumor RNA-transfected dendritic cells. Ann Surg 235: 540-549
[20] Osman Y, Narita M, Ayres F, Takahashi M, Alldawi L, Tatsuo F, Toba K, Hirohashi T, Aizawa Y (2003) Generation of Ag-specific cytotoxic T lymphocytes by DC transfected with in vitro transcribed influenza virus matrix protein (M1) mRNA. Cytotherapy 5: 161-168
[21] Ponsaerts P, Brown JP, Van Den Plas D, Van Den Eeden L, Van Bockstaele DR, Jorens PG, Van Tendeloo VF, Merregaert J, Singh PB, Berneman ZN (2004) Messenger RNA electroporation is highly efficient in mouse embryonic stem cells: successful FLPe- and Cre-mediated recombination. Gene Ther 11: 1606-1610
[22] Ponsaerts P, Van den Bosch G, Cools N, Van Driessche A, Nijs G, Lenjou M, Lardon F, Van Broeckhoven C, Van Bockstaele DR , Berneman ZN, Van Tendeloo VF (2002) Messenger RNA electroporation of human monocytes, followed by rapid *in vitro* differentiation, leads to highly stimulatory antigen-loaded mature dendritic cells. J Immunol 169: 1669-1675
[23] Ponsaerts P, van der Sar S., Van Tendeloo VF, Jorens PG, Berneman ZN, Singh PB (2004) Highly efficient mRNA-based gene transfer in feeder-free cultured H9 human embryonic stem cells. Cloning Stem Cells 6: 211-216
[24] Ponsaerts P, Van Tendeloo VF, Berneman ZN (2003) Cancer immunotherapy using RNA-loaded dendritic cells. Clin Exp Immunol 134: 378-384
[25] Ponsaerts P, Van Tendeloo VF, Cools N, Van Driessche A, Lardon F, Nijs G, Lenjou M, Mertens G, Van Broeckhoven C, Van Bockstaele DR, Berneman ZN (2002) mRNA-electroporated mature dendritic cells retain transgene expression, phenotypical properties and stimulatory capacity after cryopreservation. Leukemia 16: 1324-1330
[26] Ponsaerts P, Van Tendeloo VF, Jorens PG, Berneman ZN, Van Bockstaele DR (2004) Current challenges in human embryonic stem cell research: directed differentiation and transplantation tolerance. J Biol Regul Homeost Agents 18: 347-351
[27] Saeboe-Larssen S, Fossberg E, Gaudernack G (2002) mRNA-based electrotransfection of human dendritic cells and induction of cytotoxic T lymphocyte responses against the telomerase catalytic subunit (hTERT). J Immunol Methods 259: 191-203
[28] Schultze JL, Michalak S, Seamon MJ, Dranoff G, Jung K, Daley J, Delgado JC, Gribben JG, Nadler LM (1997) CD40-activated human B cells: an alternative source of highly efficient antigen presenting cells to generate autologous antigen-specific T cells for adoptive immunotherapy. J Clin Invest 100: 2757-2765
[29] Smits E, Ponsaerts P, Lenjou M, Nijs G, Van Bockstaele DR, Berneman ZN, Van Tendeloo VF (2004) RNA-based gene transfer for adult stem cells and T cells. Leukemia 18: 1898-1902
[30] Steinman RM, Witmer-Pack M, Inaba K (1993) Dendritic cells: antigen presentation, accessory function and clinical relevance. Adv Exp Med Biol 329: 1-9
[31] Van den Bosch GA, Ponsaerts P, Nijs G, Lenjou M, Vanham G, Van Bockstaele DR, Berneman ZN, Van Tendeloo VF (2005) *Ex vivo* induction of viral antigen-specific CD8 T cell responses using mRNA-electroporated CD40-activated B cells. Clin Exp Immunol 139: 458-467
[32] Van Den Plas D, Ponsaerts P, van T, V, Van Bockstaele DR, Berneman ZN, Merregaert J (2003) Efficient removal of LoxP-flanked genes by electroporation of Cre-recombinase mRNA. Biochem Biophys Res Commun 305: 10-15
[33] Van Tendeloo VF, Ponsaerts P, Lardon F, Nijs G, Lenjou M, Van Broeckhoven C, Van Bockstaele DR, Berneman ZN (2001) Highly efficient gene delivery by mRNA electroporation in human hematopoietic cells: superiority to lipofection and passive pulsing of mRNA and to electroporation of plasmid cDNA for tumor antigen loading of dendritic cells. Blood 98: 49-56
[34] Van Tendeloo VF, Snoeck HW, Lardon F, Vanham GL, Nijs G, Lenjou M, Hendriks L, Van Broeckhoven C, Moulijn A, Rodrigus I, Verdonk P, Van Bockstaele DR, Berneman ZN (1998) Nonviral transfection of distinct types of human dendritic cells: high-efficiency gene transfer by electroporation into hematopoietic progenitor- but not monocyte-derived dendritic cells. Gene Ther 5: 700-707
[35] Van Tendeloo VF, Van Broeckhoven C, Berneman ZN (2001) Gene therapy: principles and applications to hematopoietic cells. Leukemia 15: 523-544
[36] Van Tendeloo VF, Willems R, Ponsaerts P, Lenjou M, Nijs G, Vanhove M, Muylaert P, Van Cauwelaert P, Van Broeckhoven C, Van Bockstaele DR, Berneman ZN (2000) High-level transgene expression in primary human T lymphocytes and adult bone marrow CD34+ cells via electroporation-mediated gene delivery. Gene Ther 7: 1431-1437
[37] Zhou Y, Bosch ML, Salgaller ML (2002) Current methods for loading dendritic cells with tumor antigen for the induction of antitumor immunity. J Immunother 25: 289-303

Received: June 22, 2005
Accepted: June 24, 2005