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Research Article

Human peripheral blood mononuclear cells transfected with messenger RNA stimulate antigen-specific cytotoxic T-lymphocytes in vitro

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Abstract. The efficiency of test vaccines needs to be evaluated by quantification of the triggered cellular immune response. Usually, for these assays, autologous target cells expressing the vaccine antigen are required. In the context of messenger RNA (mRNA)-based vaccinations, the target cells used for the read-out are mRNA-transfected monocyte-derived dendritic cells (Mo-DCs). Their production typically requires samples of 100 ml blood from the patients, and limits the number of assays that can be performed. We show here that fresh peripheral blood mononuclear cells (PBMCs) can be transfected with mRNA by electroporation. Such cells are as efficient as mRNA-transfected Mo-DCs for their ability to activate memory T cells in vitro. Thus, mRNA-transfected PBMCs are a convenient replacement of mRNA-transfected Mo-DCs for the in vitro monitoring of natural or vaccine-induced immune responses.

Key words. mRNA; B lymphocyte; dendritic cell; Monocyte; T lymphocyte; MHC tetramer.

The adaptive T cell immune response participates in the control of viral spreading and tumor growth. Thus, several anti-viral and anti-tumor therapies based on the induction of an acquired T cell immunity in patients are being developed. Whether peptides [1], proteins, cell lysates, exosomes [2], DNA [3] or messenger RNA (mRNA) [4–7] are the active component of such new vaccines and whether the molecules are injected directly [8] or loaded on antigen-presenting cells (APCs) such as dendritic cells (DCs) [9, 10], the measurement of the induced cellular immune response requires in vitro cultures where autologous cells or major histocompatibility cell (MHC)-matched allogenic cells are used as targets. In the context of the promising therapies that use mRNA transfected monocyte-derived dendritic cells (Mo-DCs) as a vaccination vector [5, 11, 12], the detection of the triggered immune response by cytotoxic, proliferation or ELISpot assays requires mRNA-transfected autologous target cells. In previous studies, Mo-DCs have been used for this purpose. Although MHC class I fluorescent tetramers are very sensitive tools to detect specific cytotoxic T cells (in the case of patients expressing at least one HLA class I molecule for which epitopes of interest are known), the amplification of memory cells prior to the staining is often required to detect rare T lymphocytes of interest [13]. The limiting factor in such vaccination/read-out protocols, especially when patients suffer from aplasia or from blood malignancies, is to obtain enough blood to generate the vaccine and the target cells needed for the read-out. Indeed, the production of 10 million Mo-DCs

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which is the amount needed for a vaccination dose and for a read-out usually requires about 100 ml of blood. Since the relatively small amount of blood that can be obtained from patients is a limit for the reliability and reproducibility of the read-out methods performed during clinical trials, we searched for an alternative to Mo-DCs as mRNA-transfected target cells. We tested whether mRNA-transfected whole fresh peripheral blood mononuclear cells (PBMCs) can be used to recall immune responses in vitro. We describe here a method that allows the transfection of mRNA in PBMCs by electroporation. This results in the expression of the new gene product in all blood cell types analyzed and in the capacity of the PBMCs to present MHC-associated epitopes derived from the mRNA-encoded antigen.

Materials and methods

Messenger RNA

All the mRNAs used in this study were RNActive constructs produced by CureVac GmbH, Tübingen (**www.CureVac.com**). RNActive has a 5′Cap structure and a 3′ poly-A tail (70 As). They contain the open reading frame coding for the protein of interest (luciferase, influenza matrix, melan-A or mucin-1) flanked by untranslated regions (UTRs) from the very stable globin mRNAs.

Preparation of PBMCs

PBMCs were obtained from healthy HLA-A*0201-positive donors by Ficoll gradient separation. They were washed three times with phosphate-buffered saline (PBS).

Generation of DCs

The PBMCs were re-suspended at a concentration of 100 million in 10 ml of X-Vivo 15 medium (Bio Whittaker, Verviers, Belgium). Ten milliliters of the cell suspension was incubated in a lying 75-cm^2 tissue culture flask at 37°C for 2 h. The non-adherent cells were removed and adherent cells were cultured in X-Vivo 15 medium supplemented with 100 ng/ml granulocyte/macrophage-colony-stimulating factor (GM-CSF; Leukomax; Novartis Pharma, Basel, Switzerland) and 40 ng/ml interleukin-(IL)-4 (R&D Systems, Wiesbaden, Germany). On day 6, the cells were harvested, counted and analyzed by flow cytometry (usually ca 50% of the cells expressed the immature dendritic cell-specific markers CD1a+, CD86low).

Peptide pulsing of PBMCs/DCs

Where indicated, PBMCs and DCs were pulsed for 1 h with 1 μg/ml of the HLA-A*0201 peptide from the Influenza Matrix M1 protein (GILGFVFTL) or from the MART-1/melan-A (EAAGIGILTV) antigens. Cells were washed three times with X-Vivo 15 and then co-incubated with autologous defrosted PBMCs.

Electroporation of DCs and PBMCs

PBMCs and DCs were electroporated with the EasyjecT Plus (Peqlab, Erlangen, Germany). Per transfection, 4×10^6 cells in 200 μl Opti-MEM medium (Invitrogen, Karlsruhe, Germany) were transfected in a 0.4-cm gap electroporation cuvette (Peqlab) with 10 μg of mRNA. Settings for the electroporation were: voltage of 300 V, capacitance of 150 μF, resistance of 1540 Ohm and pulse time of 231 ms. After transfection, the cells were immediately transferred into pre-warmed X-Vivo 15 medium.

Co-culture of the electroporated cells with responding cells

Except if otherwise indicated, the electroporated PBMCs or DCs were activated by overnight culture in medium containing 100 ng/ml LPS (Sigma-Aldrich, Taufkirchen, Germany) and 2.5 ng/ml tumor necrosis factor (TNF)-α (R&D Systems, Wiesbaden, Germany). Then, the cells were washed three times with X-Vivo 15 medium and distributed in this medium at a concentration of 2 million per milliliter in wells of a 24-well plate. The same number of autologous defrosted PBMCs was added in each well. On day 4 of the culture, 10 U/ml IL-2 (R&D Systems) and 5 ng/ml IL-7 (R&D Systems) were added to the cells. If necessary, cells were re-stimulated on day 6 and day 12 with autologous mRNA-electroporated PBMCs. In this case after electroporation, PBMCs were irradiated (28 Gy) and added to the cytotoxic T lymphocyte (CTL) culture (2 million/well).

Tetramer staining

At day 6 of culture, cells were stained with HLA-A*0201 tetramers containing the immunodominant HLA-A*0201 epitope from influenza matrix M1 or MART-1/melan-A. The cells were first stained with a fluorescein isothiocyanate (FITC)-conjugated anti-CD4 antibody and a peridinin-chlorophyll-protein complex (PerCP)-conjugated anti-CD8 antibody (Becton Dickinson, Heidelberg, Germany) in a PBS buffer supplemented with 0.5% bovine serum albumin and 2 mM EDTA for 30 min. The cells were washed once with PFEA [PBS with 2% fetal calf serum (FCS), 2 mM EDTA, 0.01% sodium azide]. Then, the adequate phycoerythrin (PE)- or allophycocyanine (APC)-labeled MHC class I tetramer was added to a final concentration of 5 μg/ml in PBS/50% FCS. The cells were further incubated for 30 min at 4°C before being washed with PFEA, fixed with PBS containing 1% formaldehyde and analyzed by four-color flow cytometry.

Intracellular cytokine staining

At day 6 of the culture, cells were incubated for 5 h in X-Vivo 15 medium with 0.66 μl/ml GolgiStop (BD Pharmingen, Heidelberg, Germany) in 96-well culture plates in the presence of 1 μg/ml of synthetic peptide.

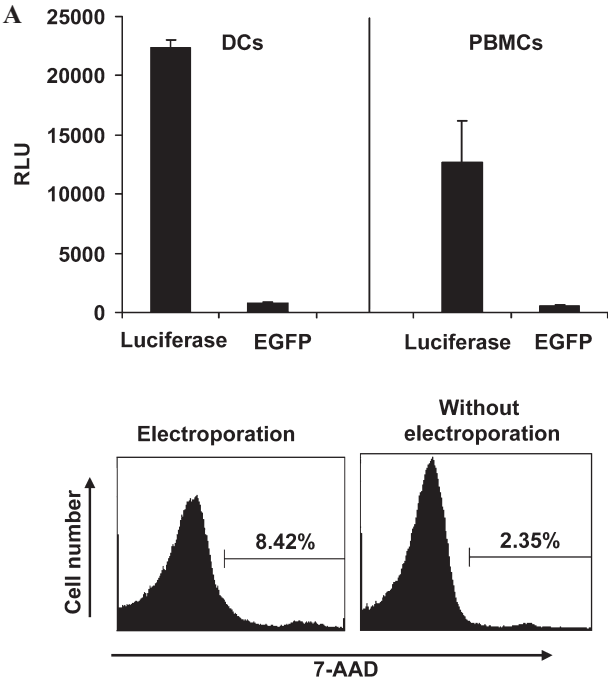

Figure 1. Expression of reporter molecules in mRNA-transfected PBMCs. Immature dendritic cells (DCs) or PBMCs were transfected with RNActive-coding firefly luciferase or enhanced green fluorescent protein (EGFP). (*A*) The luciferase activity was measured in the cell lysates 6 h after transfection. RLU, relative light units. This is the crude value given by the luminometer. The percentage of dead cells among PBMCs is shown in the histograms (7-AAD staining of the PBMCs 6 h after electroporation).

Then, cells were pelleted by centrifugation, fixed and permeabilized with 150 µl/well Cytofix/Cytoperm (BD Pharmingen) for 20 min at 4 °C. The cells were washed twice with washing buffer (BD Pharmingen) and stained with a FITC-conjugated anti-CD4 antibody, a PerCP-conjugated anti-CD8 antibody and a PE-conjugated anti-TNF-α antibody (all from BD Pharmingen).

Detection of luciferase activity

Six hours after electroporation of PBMCs with RNActive coding for luciferase, 4 million cells were lysed with 400 µl of lysis buffer (25 mM Tris-PO_4, 2 mM EDTA, 10% glycerol, 1% Triton-X 100, 2 mM DTT). Supernatant (50 µl) was mixed with 400 µl luciferin buffer (25 mM glycylglycin, 15 mM $MgSO_4$, 5 mM ATP, 62.5 µM luciferin). Then the luminescence was measured on a luminometer (Lumat LB 9507; Berthold Technologies Bad Wildbad, Germany).

Test of viability after electroporation

Six hours after electroporation, cells were washed twice with PBS. Then, 7-amino-actinomysin-(7-AAD) staining (BD Pharmingen) was performed. 100,000 cells were re-suspended in 150 µl PBS + 1% FCS and stained with 3.2 µl 7-AAD. Fluorescence was measured immediately by flow cytometry.

Results

Fresh PBMCs can be transfected by mRNA using electroporation

We tested several different protocols of electroporation (in different media, using voltage from 200 to 600 V and capacitance from 150 to 1500 µF, single or double pulses), and found that PBMCs mixed with mRNA coding for luciferase (luciferase-encoding RNActive, see Materials and methods) and electroporated at 300 V and 150 µF express luciferase which can be detected in cell lysates 6 h after the pulse (fig. 1A). In comparison to the elec-

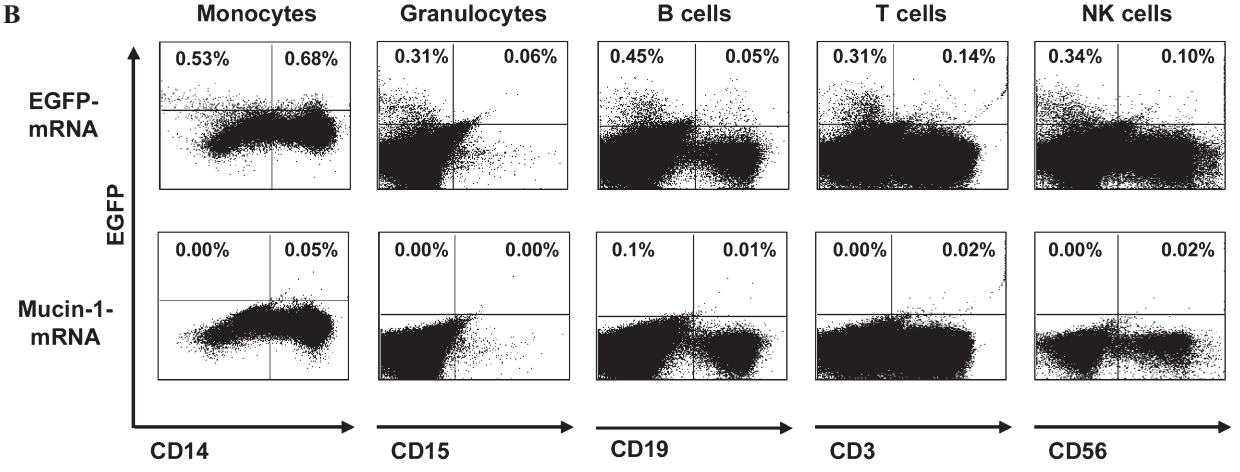

Figure 1. (*B*) EGFP expression was studied by FACS analysis after antibody staining of the mRNA-transfected PBMCs 6 h after transfection (electroporation of mRNA coding for mucin-1 is used as a negative control). In both cases, the reporter protein encoded by the RNActive can be detected. This shows that the conditions of electroporation allow transfection of the cells by mRNA. These experiments were repeated several time using PBMCs from five different donors.

troporation of immature Mo-DCs in the same conditions, the luciferase activity measured in PBMCs is usually lower. Cell death induced by this electric shock is low (ca 9% as indicated by 7-AAD staining; fig. 1A). To identify the cell type(s) which are transfected, we used RNActive coding for enhanced green fluorescent protein (EGFP) and measured the FL1 fluorescence of T cells, B cells, granulocytes, monocytes and natural killer (NK) cells. As shown in figure 1B, a weak but detectable expression of EGFP compared to the negative control, PBMCs electroporated in the presence of a mucin-1-coding RNActive, could be detected in 0.1% up to 1.5% of the PBMCs (as seen when summing-up the percentages of EGFP-positive cells in each cell type) depending on the experiment, i.e. in different donors and blood preparations. A small fraction of cells among all studied sub-populations detectably expressed EGFP. However, in these conditions of pulse, NK cells, T cells and monocytes seemed to be preferentially transfected. Several electroporation conditions, including double pulses, led to a higher number of EGFP-positive cells; however, the resulting cellular mortality was also increased (data not shown). We concluded that with our electroporation device, 300 V and 150 µF are the optimal conditions for the transfer of mRNA into PBMCs. Knowing that passive transfer of mRNA in DCs by co-incubation of DCs and mRNA for 45 min at 37 °C leads to epitope presentation but not to a detectable expression of the exogenous mRNA-encoded protein [5], we expected that the mRNA-transfected PBMCs in which protein expression can be detected will be efficient enough in presenting MHC-associated epitopes to T cells. To verify this hypothesis, we used mRNA-transfected PBMCs as target cells for antigen-specific cytotoxic T cells.

Messenger RNA-transfected PBMCs present MHC-class I-associated epitopes derived from the mRNA-encoded protein

Fresh PBMCs from HLA-A*0201 healthy donors were electroporated with RNActive coding for the influenza matrix protein M1 (Flu M1) or coding for EGFP as a negative control. The electroporated cells were then kept overnight in culture medium with lipopolysaccharide (LPS) and TNF-α or without danger signals that can activate directly and indirectly (through the release of cytokines by cells stimulated by LPS and TNF-α) many cell types, including monocytes and B lymphocytes. Such treatment should increase the cell surface expression of MHC antigens and activation molecules (MHC class I, MHC class II, CD80, CD83, CD86) on several cell types rendering them more potent in antigen presentation. Then, the electroporated PBMCs were co-cultured with

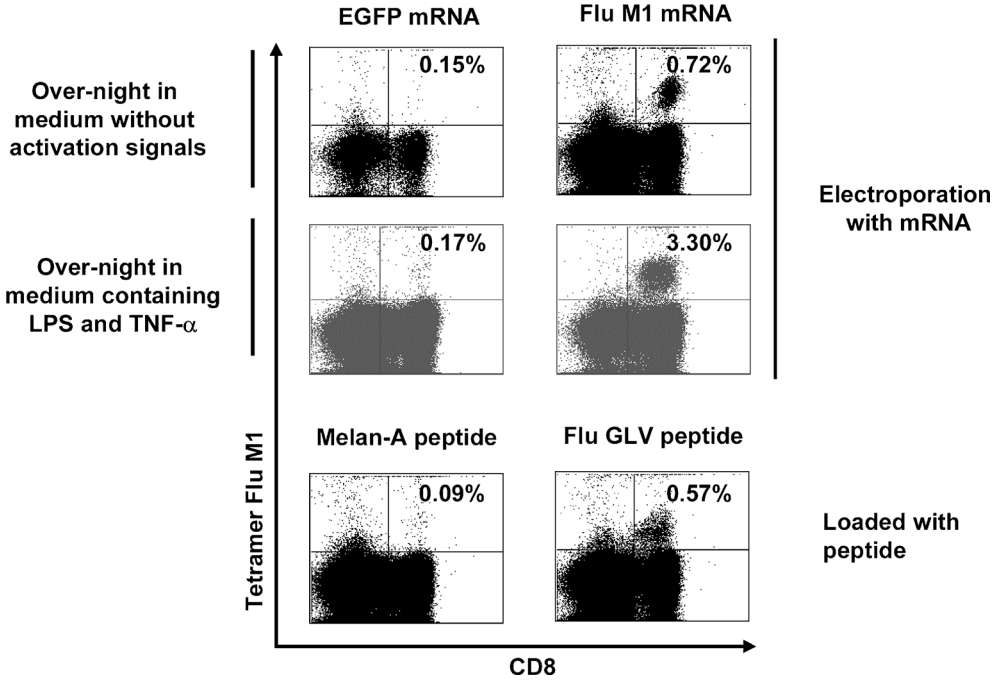

Figure 2. PBMCs transfected with mRNA are APCs. PBMCs transfected with EGFP- (left panel) or Flu M1-coding RNActive (right panel) were (the two dot plots in the middle) or were not (the two upper dot plots) stimulated overnight with LPS and TNF-α and then co-cultured with autologous defrosted PBMCs. As controls (the two lower dot plots on the right), the stimulating PBMCs were pulsed with peptides corresponding to the MART-1/melan-A (left panel) or the Flu M1 (right panel) immunodominant HLA-A*0201 epitopes and co-cultured with autologous defrosted PBMCs. At day 6 of culture, the cells were stained with antibodies and a fluorescent MHC class I tetramer specific for Flu M1 HLA-A*0201-restricted T cells and analyzed by FACS. The experiment was repeated twice with two different donors and gave comparable results.

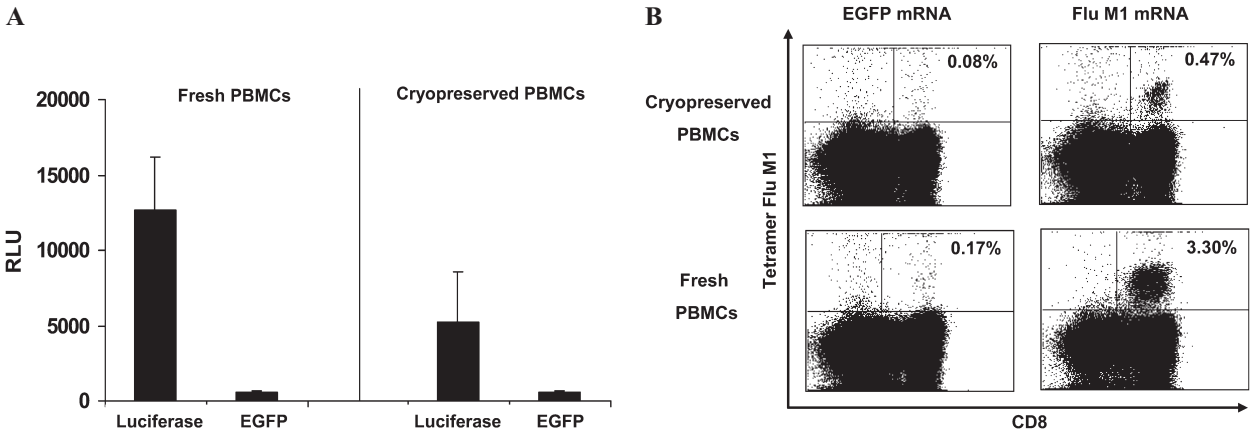

Figure 3. Thawed PBMCs can be transfected with mRNA. (*A*) Fresh or defrosted PBMCs were electroporated with RNActive coding for luciferase. Six hours after transfection, cells were lysed and the luciferase activity was measured. RLU, relative light units. This is the crude value given by the luminometer. The graph shows the mean and standard deviation of the results obtained in three independent transfections. (*B*) Fresh (lower dot plots) or defrosted (upper dot plots) PBMCs were used for electroporation with RNActive coding for EGFP (left panel) or Flu M1 (right panel). The electroporated cells were co-cultured with autologous thawed PBMCs for 1 week and stained with antibodies and a fluorescent MHC class I tetramer specific for Flu M1 HLA-A*0201-restricted T cells and analyzed by FACS. Although they are less efficient than fresh PBMCs, thawed electroporated PBMCs can stimulate the proliferation of Flu M1-specific CTLs. The experiments were repeated twice.

an equal amount of autologous PBMCs that had been stored at –80 °C. One week later, the cells were stained with a fluorescent HLA-A*0201 tetramer folded around the immunodominant epitope from the Flu M1 protein (peptide GILGFVFTL [14]). The results presented in figure 2 show that Flu M1-specific cytotoxic T cells contained in defrosted PBMCs proliferated when incubated with autologous PBMCs transfected with Flu M1-encoding RNActive but not with EGFP-encoding RNActive. As positive control, we used the defrosted cells incubated for 1 week with peptide-pulsed autologous PBMCs. In the presence of the GILGFVFTL peptide but not in the presence of an irrelevant peptide (MART-1/melan-A HLA-A*0201 epitope AAGIGILTV [15]), we recorded the specific proliferation of anti-influenza CTLs (fig. 2, lower panels: 0.57% of the gated cells were stained with the Flu M1 tetramer as opposed to 0.09% positive cells in the MART-1/melan-A peptide culture). In independent experiment we found that in the absence of activation of the target cells by a danger signal, the proliferation of Flu M1-specific cytotoxic T cells was weaker. These experiments show that mRNA-transfected fresh PBMCs express efficiently the mRNA-encoded protein-derived MHC class I epitopes to cytotoxic T cells and that antigen presentation by such cells is increased by a pre-treatment with immunostimulating molecules.

Fresh as well as cryopreserved PBMCs can be transfected with mRNA

Since the study of the cellular immune response is often done using frozen PBMCs preserved in liquid nitrogen, we tested whether such cells are suitable for mRNA electroporation. As shown in figure 3A, the expression of luciferase could be detected in electroporated thawed PBMCs although it was lower than that observed in electroporated fresh PBMCs. Similarly, when defrosted PBMCs were electroporated, their capacity to re-stimulate memory T cells was reduced compared to mRNA-electroporated fresh PBMCs: 0.47% of tetramer-positive cells when thawed PBMCs were used for transfection compared to 3.3% when fresh PBMCs were used for transfection. Aside from the lower antigen expression, a reduction in the capacity of the electroporated defrosted cells to stimulate CTLs may also be due to the fragility of thawed PBMCs. In particular, some of the professional APCs contained in PBMCs, i.e. monocytes, plasmacytoid DCs (pDCs) and myeloid DCs (mDCs), may be even more damaged than lymphocytes by the freezing-thawing process and impair the overall quality of the stimulation that is provided by mRNA-transfected cells to the CTLs. Nevertheless, we show that cryopreserved PBMCs can be utilized for mRNA transfection by electroporation and subsequently serve as stimulating cells, which is important for practical reasons.

PBMCs transfected with mRNA efficiently stimulate only memory cytotoxic T cells

Knowing that some professional APCs such as pDCs, mDCs, B cells and monocytes are contained in PBMCs, we studied the T cell priming (activation of naive cells) versus T cell re-stimulation (activation of memory cells) capacity of mRNA-transfected PBMCs. In the context of the re-stimulation capacity, we used again the RNActive coding for the Flu M1 protein and studied the expansion of specific cells with the HLA-A*0201 tetramer folded around the immunodominant GILGFVFTL epitope. For

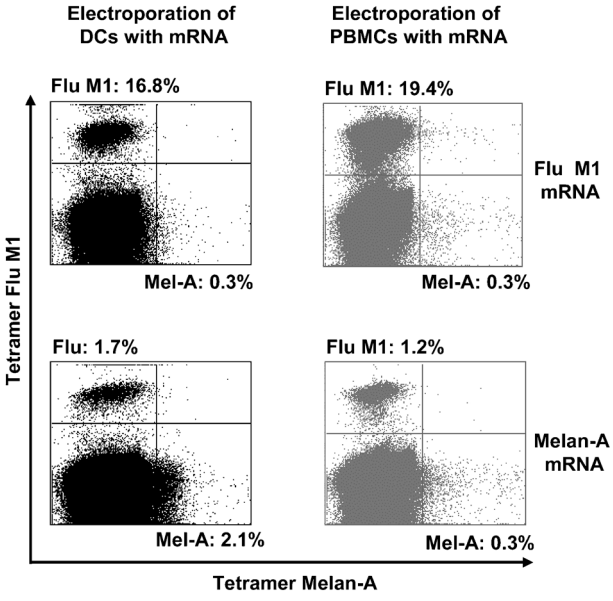

Figure 4. PBMCs transfected with mRNA cannot prime naive CTLs. DCs (left panels) or PBMCs (right panels) were transfected with RNActive coding for the Flu M1 protein (upper dot plots) or for the MART-1/melan-A antigen (lower dot plots). The electroporated cells were co-cultured with autologous defrosted PBMCs for 3 weeks (mRNA-electroporated irradiated autologous PBMCs were added in the cultures once a week) and stained with antibodies, a PE-conjugated MHC class I tetramer specific for Flu M1 HLA-A*0201-restricted T cells and an APC-conjugated MHC class I tetramer specific for MART-1/melan-A HLA-A*0201-restricted T cells and analyzed by FACS. As opposed to DCs that can stimulate the proliferation of Flu M1- and MART-1/melan-A-specific T cells, transfected PBMCs can trigger the proliferation of memory (Flu M1-specific) but not naive (MART-1/melan-A-specific) cytotoxic T cells. In the context of memory T cell proliferation (Flu M1-coding mRNA), mRNA-transfected PBMCs are as efficient as mRNA-transfected DCs.

the T cell priming activity, we used RNActive coding for the tumor antigen MART-1/melan-A [15] for which a high number of naive T cell precursors are contained in the blood of most HLA-A*0201-positive healthy donors. As a positive control, we used mRNA-transfected mature autologous DCs for the stimulation. As shown in figure 4, mRNA-transfected PBMCs are as efficient as mRNA-transfected DCs in stimulating the proliferation of memory T cells specific for the Flu M1 protein. In contrast, the proliferation of MART-1/melan-A-specific T cells is triggered by mRNA-transfected DCs (2.1% of MART-1/melan-A tetramer-positive cells) but not by mRNA-transfected PBMCs (0.3% of MART-1/melan-A tetramer-positive cells, which is similar to the background obtained when staining with the MART-1/melan-A tetramer in vitro-cultured PBMCs co-incubated with influenza matrix M1-transfected PBMCs or DCs). Similar results were obtained with different healthy donors from the blood bank. These results suggest that in spite of LPS and TNF-α activation of APCs contained in PBMCs such as mDCs, pDCs, B cells and monocytes, no efficient priming of naive T cells is obtained in vitro using mRNA-transfected PBMCs, at least not with our present culture conditions.

Stimulation with mRNA-transfected PBMCs generates armed effector cells

The proliferation of antigen-specific T cells is not always accompanied by the development of effector capacities and is actually sometimes associated with functional anergy. In the above experiments, we studied the stimulation of antigen-specific T cells by detecting their expansion using MHC class I tetramer staining. Thus, we cannot judge the effector capacities of re-stimulated memory T cells. In figure 5 we show the status of the specific T cells that proliferated in vitro in contact with mRNA-transfected PBMCs. Cells that were co-cultured for 1 week with Flu M1-coding RNActive-transfected PBMCs were pulsed with a synthetic GILGFVFTL peptide. The peptide and an inhibitor of secretion were directly added in the medium. After 5 h of culture and after fixation and permeabilization of the cells, we studied the presence of TNF-α by intracellular staining. Many Flu M1-specific T cells could be detected in the in vitro-expanded T cells when using Flu M1 MHC tetramer or intracellular cytokine staining.

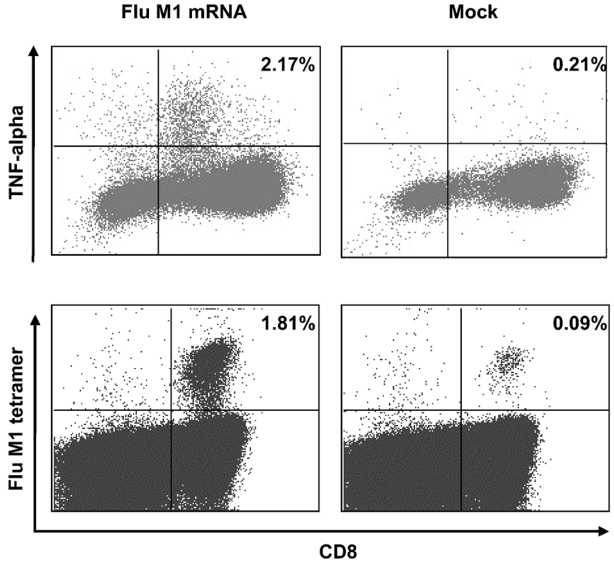

Figure 5. Cytotoxic T cells specifically expanded by co-culture with mRNA-transfected PBMCs are effector cells. Intracellular cytokine staining (upper dot plots) or fluorescent MHC class I tetramers (lower dot plots) were used to detect Flu M1-specific T cells in PBMCs co-cultured for 1 week with Flu M1-coding RNActive- (left panel) or mock transfected (right panel) PBMCs. For intracellular staining, the cells were cultured for 5 h with the Flu M1 dominant HLA-A*0201 epitope in the form of a synthetic peptide before fixation/permeabilization and antibody staining. The experiment shows that the Flu M1-specific cytotoxic T cells that expanded in vitro in contact with electroporated PBMCs are producing TNF-α when stimulated with the adequate peptide and are therefore effector cells. This experiment was reproduced twice in three different donors.

Since a short-term peptide re-stimulation was used to trigger cytokine expression in influenza-matrix 58-66-specific CTLs, the T cell receptor and the co-stimulation molecules (CD8 for example, as seen in fig. 5) are reduced in the peptide-specific CTLs [16]. This experiment shows that T cells expanded in vitro upon recognition of mRNA-transfected PBMCs are armed effector cells which respond to peptide re-stimulation by cytokine release.

Discussion

Whether transfected in vitro into DCs [5], delivered intradermally by gene gun [17], self-replicative [6] or stabilized [4, 7], mRNA has been successfully used as a vector for vaccination in several animal models (for a review on mRNA-based vaccines see Pascolo [18]). Moreover, immunization using mRNA-transfected DCs was recently reported to be efficient in tumor patients [11, 12]. In these studies, the authors used cryopreserved PBMCs isolated at different time points from the blood of patients to study the immune response induced by vaccination. Usually, in such in vitro studies, autologous DCs prepared from PBMCs are used as APCs in ELISpot assays for example. A major limit in the reproducibility of these immunomonitoring assays is the amount of blood that can be collected from the vaccinated patients. Since about 10 million DCs are obtained from 100 ml of fresh blood, the in vitro read-out cannot be reproduced many times. Moreover, if DCs are used as APCs, not only re-call responses but also primary responses are recorded. What is desired, however, is the quantification of re-call responses. Consequently, the autologous target cells used in the assays (ELISpot or in vitro amplification of the specific T cells for cytotoxic assays or sensitive tetramer staining) should not be DCs. As an alternative to Mo-DCs, we evaluated a faster and easier approach, the direct transfection of mRNA into PBMCs. To start with, we tested different liposome- or non-liposome-based transfection reagents. None of them allowed a reproducible transfection of PBMCs with EGFP- or luciferase-coding mRNA and all were quite toxic for the cells (data not shown). In contrast, using electroporation, we could find conditions that led to a detectable expression of the protein encoded by the exogenous mRNA and a very low toxicity due to the transfection (fig. 1). Such mRNA-transfected cells present MHC class I epitopes to CTLs as documented by the specific expansion of influenza-specific CTLs upon co-culture with the Flu M1-encoding mRNA-transfected PBMCs (fig. 2). Since both fresh and thawed cells can be efficiently electroporated with mRNA and re-stimulate specific cytotoxic T cells, we anticipate that this method will be widely used to study in vitro the immune response triggered in patients vaccinated with vectors that encode full-length proteins (recombinant virus-, DNA- or mRNA-based vaccines). Using this technology, we managed to detect the activation of antigen-specific CD4-positive T helper cells (data not shown). The appropriate parameters, for example, cytokine production, proliferation and T cell receptor down-regulation, and methods are being optimized to turn the mRNA transfection in PBMCs into a quick and reliable technique for monitoring the antigen-specific cytotoxic and helper T cell responses present in blood. Furthermore, the possibility of transfecting mRNA in PBMCs may lead to a new vaccination method. Up to now, DCs generated in vitro are used for vaccination with the theoretical background that such cells can prime an immune response through co-stimulation and cytokine release. Meanwhile, some other APCs such as CD40-activated B cells obtained after 1 week of in vitro culture, were also shown to be permissive to mRNA transfection by electroporation and efficient in priming T cells [19]. These methods to generate autologous APCs require extensive in vitro culture of patient cells. Consequently, these therapies are associated with high costs and need the implementation of complicated structures, i.e. good manufacturing practice facilities, fast transport of the blood or DCs from the patient to the site of culture and so on. We propose mRNA-transfected PBMCs as an alternative to mRNA-transfected in vitro-generated professional APCs as a vaccination method. The fact that mRNA-transfected PBMCs cannot prime T cells in vitro does not indicate that the same will be true in vivo. Indeed, there is a recent report that the triggering of an immune response after injection of mRNA transfected DCs may be actually due to cross-priming and not (only) to the anticipated direct activation of naive T cells by the transfected APCs [20]. Since mRNA is a known danger signal [21–24], it may also act as an adjuvant in such mRNA-transfected PBMC-based vaccines. According to these results, we expect that autologous PBMCs transfected in vitro with mRNA coding for an antigen may be efficient in priming an immune response. Vaccination studies in adequate animal models with autologous mRNA-transfected PBMCs may lead to the development of a new mRNA-based vaccination strategy that would be easier and less expensive than therapies using Mo-DCs or activated B cells.

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