



Expression of the nlsLacZ gene in dendritic cells derived from retrovirally transduced peripheral blood CD34⁺ cells

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ABSTRACT

Background and Objective. Gene transfer and expression of exogenous genetic information coding for an immunogenic protein in antigen presenting cells (APCs) can promote an immune response. This was investigated by retroviral transfer of a marker gene into CD34⁺ derived APCs.

Design and Methods. To achieve long term expression of a specific transgene in APCs, G-CSF mobilized peripheral blood CD34⁺ cell populations were retrovirally transduced with the bacterial nlsLacZ, a marker gene used here as a model, in the presence of IL-3, IL-6, GM-CSF and SCF prior to being induced to differentiate into dendritic and macrophage cells by GM-CSF and TNF- α .

Results. Addition of IL-4 was found to induce dendritic differentiation preferentially by inhibiting proliferation and differentiation of the macrophage lineage. As assessed by X-Gal staining, LacZ gene expression was observed in cells from both the dendritic lineage (CD1a⁺/CD14⁻) which still exhibits the highest immunostimulatory activity in mixed lymphocyte reaction and from the macrophage lineage (CD1a⁻/CD14⁺).

Interpretation and Conclusions. This study sets out the possibility of transducing dendritic and macrophage progenitors present in the CD34⁺ cell population and in using a marker gene such as nlsLacZ to study gene expression in antigen presenting cell compartments.

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Key words: dendritic cells, LacZ, retroviral vector, CD34 cells

Dendritic cells (DCs) are a heterogeneous family of antigen presenting cells (APCs) which interact with T lymphocytes to trigger immune responses.¹ Several reports have shown that, *in vitro*, DCs can capture, process, and present antigens.^{2,3} These cells are potent stimulators in allogeneic mixed leukocyte reactions⁴ and are capable of activating naive CD4⁺ T cells in an antigen specific manner.⁵ Dendritic cells also stimulate the proliferation of allogeneic CD8⁺ T cells *in vitro*.^{6,7}

Inoculation of mice with small numbers of allogeneic DCs⁸ or with autologous DCs pulsed with peptide, whole protein⁹ or transfected with DNA,¹⁰ induces strong CTL responses. Due to their immunostimulatory activity, DCs would be interesting vehicles for presenting transgenic products in order to induce specific immune responses.

DCs can be generated from mature peripheral blood mononuclear cells in the presence of cytokines such as granulocyte-macrophage colony-stimulating factors (GM-CSF) and tumor necrosis factor- α (TNF- α), potentially from a CD33⁺ CD11b⁺ CD14⁻ precursor cell common to both monocyte-macrophage and dendritic lineages.¹¹ Preferential differentiation along the dendritic lineage could be achieved by addition of interleukin 4 (IL-4).^{11,12} Such mature cells can be used as the target for gene transfer using viral vectors as has already been achieved with the retroviral transfer of the LacZ gene or the interleukin-7 gene,^{13,14} and, for example, with adenoviral transfer of the green fluorescent protein gene,¹⁵ or non viral delivery strategies with transfection of tumor associated antigen genes.^{16,17} However, the DC differentiated compartment does not exhibit self-renewal ability and has a relatively low proliferative capacity, thus not allowing long-term expression of the transgene when, for example, long-term antigen presentation is desired. Therefore transfer of a putative therapeutic gene by an integrative vector which allows persistence of the new genetic information in the progeny should be envisaged at the level of dendritic progenitors. Such progenitors are present in the CD34⁺ cell population derived from cord blood,¹⁸ bone marrow,¹⁹ or mobilized peripheral blood.²⁰ When derived *in vitro* from CD34⁺ progenitors, DCs expressing several cell surface markers, including CD1a, CD40, CD80, CD83, CD86, CD4, were described.^{11,21} These combinations of cell surface markers have already been shown to sort out or to characterize DCs, particularly CD1a combined with HLA-DR, CD80, CD86, and the absence of CD14.

Gene transfer and integration of a transgene into a host genome which promotes maintenance of the transgene of interest in the progeny of transduced cells can be attempted by using retroviral vectors.²²⁻²⁴ Efficient retroviral gene transfer into primitive hematopoietic stem cells still remains a challenge, but

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numerous works have demonstrated the possibility of achieving this goal with progenitor cell populations and in obtaining gene expression in myeloid and lymphoid cells. In this context, marker genes, such as nlsLacZ and cell surface markers, proved to be useful in order to assess gene transfer and expression in target cells.²⁵⁻²⁹ It is interesting that adenoviral vectors, which remain episomal, can also be used to obtain gene transfer into CD34⁺ derived DC progenitors for applications in which high transgenic expression for limited periods of time is required.³⁰

In this study, CD34⁺ cells obtained from peripheral blood of cancer patients after mobilization by recombinant human granulocyte colony-stimulating factor (G-CSF) were co-cultivated with a nlsLacZ containing retroviral vector producing cell line and induced to proliferate and differentiate in the presence of stem cell factor (SCF), GM-CSF and TNF- α , with or without IL-4. The nlsLacZ marker gene encodes for a nuclear localized β -galactosidase whose activity can be detected as a blue-indigo staining with the chromogenic substrate 5-bromo-4-chloro-3-indolyl- β -D-galactoside (X-Gal) and used to tag and track genetically modified cells.^{31,32} Retroviral gene transfer and expression of the nlsLacZ gene has proven not to be deleterious for human hematopoietic cells.³³⁻³⁵ We demonstrated in this study that induction of mobilized peripheral blood CD34⁺ cells to differentiate into DC cells or macrophages can be combined with retroviral transduction of progenitors using a co-cultivation strategy and that expression of the nlsLacZ gene can be used to monitor gene expression in subsequent genetically modified cells.

Design and Methods

CD34⁺ cell selection

Cell samples were obtained, after informed consent, from aphereses collected in cancer patients stimulated with 300 mg or 600 mg recombinant human G-CSF (Filgrastim, AMGEN, Thousand Oaks, CA, USA) as reported elsewhere.³⁶ Mononuclear cells were obtained by density gradient centrifugation and were depleted of adherent cells by overnight incubation on flat bottom flasks in RPMI-1640 medium (GIBCO, Grand Island, NY) with 10% fetal calf serum (FCS) (Biowhittaker, Walkersville, MD, USA), at 37°C in a 5% CO₂ atmosphere. Viability was evaluated by trypan blue exclusion and was always greater than 95%. CD34⁺ cells were purified by positive immunoselection according to the manufacturer's recommendations (Magnetic Activated Cell Sorting, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) and CD34⁺ enrichment was evaluated by flow cytometry (FACScan, Becton Dickinson, San José, CA, USA) by using phycoerythrin (PE)-conjugated anti-CD34 MoAb (HPCA-2; Becton Dickinson). Cells were then frozen in fetal calf serum containing 10% DMSO.

Generation of dendritic cells from PBMC CD34⁺

DCs were generated using a slightly modified method, as described by Caux *et al.*¹⁸ to generate DC from umbilical cord blood. DC cultures were initiated from freeze-thawed PBMC CD34⁺ cells at 2×10⁴ cells/mL (4×10⁴ cells/wells) in a 24-well culture plate in the presence of 100 ng/mL recombinant human GM-CSF (Schering-Plough, Dardilly, France), 2.5 ng/mL recombinant human TNF- α (Schering-Plough) and 100 ng/mL recombinant human stem cell factor SCF (Amgen) in RPMI-1640 medium supplemented with 1% penicillin-streptomycin (GIBCO) and 10% FCS at 37°C in a fully humidified incubator with a 5% CO₂ atmosphere. The cultures were then split every 4 to 5 days with medium containing fresh factors and the cells were seeded at 1 to 3×10⁵ cells/mL. SCF was added only for the first three days. Both cells in supernatant and adherent cells recovered using a 5 mM EDTA solution were counted on the days indicated. Cultures were initiated in the presence or absence of 200 U/mL recombinant human IL-4 (Schering-Plough).

Gene transduction protocol

Retroviral transduction was performed as follows: CD34⁺ cells were co-cultivated with A7/21, a Psi-Crip derived MFG-S-nls-LacZ producing cell line (developed in collaboration with Somatix Therapy Co., Alameda, USA) which exhibits an average viral titer of 2×10⁶ viral particles per mL. Two ×10⁵ producer cells were treated with mitomycin C (Sigma) at 10 mg/mL for 3 hours and washed three times with PBS 1X. Then 2×10⁵ CD34⁺ cells, incubated for 24h with SCF (100 ng/mL) and GM-CSF (100 ng/mL), were plated on a confluent layer of the packaging cell line for 72h in RPMI-1640 supplemented with 10% FCS, 10 ng/mL IL-3, 10 ng/mL IL-6, 100 ng/mL SCF, 100 ng/mL GM-CSF and 4 mg/mL polybren. After this period, non-adherent cells were gently harvested from the plate and seeded into new dishes in the absence of producer cells. After the transduction, cells were cultivated in liquid culture with GM-CSF (100 ng/mL), TNF- α (2.5 ng/mL) and SCF (100 ng/mL) in RPMI-1640 medium (GIBCO) supplemented with 10% FCS, 1% penicillin-streptomycin, in the presence or absence of IL-4.

Flow cytometric analysis

Immunophenotypes were identified by FACS analysis using monoclonal antibodies coupled to rhodamine (RHO): anti CD1a RHO (T6-RD1, Immunotech-Coulter, Marseille, France), mouse IgG1 RHO (IgG1-RD1, Immunotech-Coulter), or coupled to fluorescein isothiocyanate (FITC): IGG2a FITC (Beckton Dickinson), antiCD14 FITC (Immunotech-Coulter), anti HLA-DR FITC (Beckton Dickinson), HLA-ABC FITC (Immunotech-Coulter), IGG1 FITC (Beckton Dickinson), antiCD4 FITC (LEU 3A; Beckton Dickinson). Briefly, cells were resuspended in PBS 1X containing 2.5 % human polyvalent immunoglobulin

lins and seeded into microtiter plates (V bottom) at a final concentration of 10^5 cells/well. Cells were incubated with conjugated mAbs at saturating concentrations for 30 min at 4°C , washed twice with 150 mL PBS 1X-2.5% human polyvalent immunoglobulins, and 5mM EDTA to avoid aggregation. Fluorescence analyses were performed with a FACScan flow cytometer (Beckton Dickinson).

Isolation of dendritic and macrophage populations by FACS-sorting

Cells were labeled with anti-CD14 FITC and anti-CD1a RHO and sorted according to CD1a and CD14 expression into CD1a⁺/CD14⁻, CD1a⁺/CD14⁺, and eventually CD1a⁻/CD14⁻, CD1a⁻/CD14⁺ fractions using a FACS Vantage (Beckton Dickinson). Figure 4 shows the gating strategy used to sort these populations. Sorting was performed 6 days after TNF- α addition to the liquid culture or co-cultivation with the retroviral vector producing cell line.

Allogeneic mixed leukocyte reaction (MLR)

Peripheral blood lymphocytes (PBL) were isolated by gradient centrifugation from blood samples obtained from healthy donors. T cells were purified by a double-rosetting technique, using 2-amino-ethyl-isothiuronium-bromide (AET)-treated sheep red blood cells, followed by overnight adhesion on plastic dishes in RPMI-1640 medium containing 30% fresh, non-heated human plasma. CD4⁺ T lymphocytes were then purified by immunomagnetic depletion using a cocktail of MoAbs: CD8 (B9.11), CD14 (RM052), CD56 (T199) and CD19 (J4.119) (Immunotech-Coulter). After selection, the purity of CD4⁺ T cells was found to be higher than 85%. APCs expanded from non-genetically modified CD34⁺ PBMC were incubated with mitomycin C at a final concentration of 50 mg/mL for 30 min at 37°C and washed thoroughly. Increasing numbers (1×10^4 ,

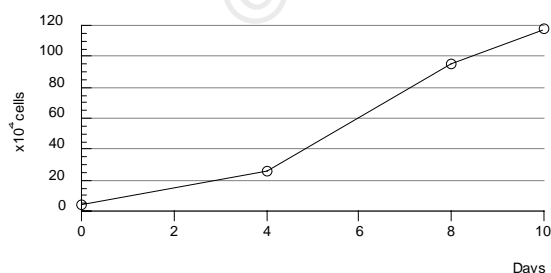


Figure 1. Growth kinetics of mobilized peripheral blood CD34⁺ cells derived-cell populations cultivated in the presence of GM-CSF and TNF- α . Four $\times 10^4$ freeze-thawed CD34⁺ cells were seeded into complete culture medium supplemented with 10 ng/mL GM-CSF, 2.5 ng/mL TNF- α , and for the first three days with 100 ng/mL SCF (c-kit Ligand). Both adherent cells and non adherent cells were collected on the days indicated and counted. Means from five independent experiments are indicated.

5×10^4 or 1×10^5) of CD34⁺ derived dendritic cells were added to 1×10^5 allogeneic CD4⁺ cells in 96-microwell tissue culture plates. After 6 days of culture in RPMI-1640 medium containing 20% FCS (Biowhittaker), cells were pulsed with ^3H -thymidine (0.5 mCi per well) for 18 hours, and incorporation of radionuclide was measured in triplicate experiments by β -scintillation spectroscopy.

Results

Heterogeneous cell populations containing dendritic cells are generated in the presence of SCF, GM-CSF and TNF- α

As indicated in Figure 1, when purified CD34⁺ cells were cultured for 10 days in liquid culture in the presence of TNF- α , GM-CSF and SCF, total cell number, including adherent and non-adherent cells, clearly increased. At day 10 a heterogeneous cell population was observed, which included non adherent cells with rounded morphology or cytoplasmic spikes peculiar to DCs along with a monolayer of large adherent flat macrophage-like cells (Figure 2a).

When characterized by the expression of CD1a and CD14 antigens, four populations were observed: (i) a CD1a⁺/CD14⁻ cell population defined as dendritic cell subset as already reported by many authors, (ii) a CD1a⁻/CD14⁺ macrophage population, (iii) cells expressing both CD1a and CD14 antigens, and (iv) a CD1a⁻ and CD14⁻ cell population. When monitored from day 3 to 10, the CD1a⁺/CD14⁻ and CD1a⁻/CD14⁺ populations were observed after day 5 whereas the CD1a⁺/CD14⁺ subset appeared later, about day 8 to day 10 (Figures 3 and 4). Concomitantly, a decrease in the percentage of the CD1a⁻/CD14⁻ population was observed. Dendritic cells and macrophages were shown to be the prominent cell populations present in cell cultures, and according to the total cell increase after ten days in culture, CD1a⁺/CD14⁻, CD1a⁻/CD14⁺ and CD1a⁺/CD14⁺ populations were found to be amplified (Figures 1 and 3).

The CD1a⁻/CD14⁻ negative population was found to increase during culture. This population, strongly positive for HLA class I antigens, did not express CD7, CD19, CD86, CD56, CD34 or GPA, but 30 to 50% of the cells were found to express CD33.

Interleukin-4 inhibits macrophage proliferation and drives differentiation towards dendritic cells

In order to decrease the percentage of macrophages derived from CD34⁺ progenitors and to increase the percentage of CD1a⁺/CD14⁻ dendritic cells during cell proliferation and differentiation, cultures were also performed in presence of IL-4, a cytokine known to inhibit macrophage proliferation. No adherent cell layer was detected in the culture when IL-4 was added to the previously described combination of cytokines. As shown in a typical experiment, cell expansion was roughly identical in the absence or presence of 200

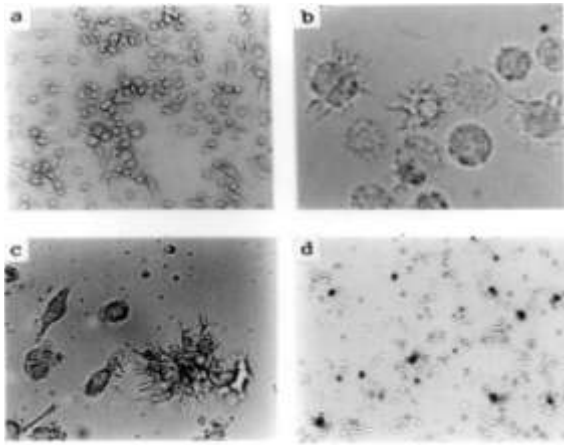


Figure 2. Photomicrographs of (a) mobilized peripheral blood CD34⁺ cells-derived cell populations cultivated in the presence of GM-CSF and TNF- α at day 11, (b) representative sorted CD1a⁺/CD14⁻ cells alone or (c) aggregated, (d) X-Gal staining of representative sorted CD1a⁺/CD14⁻ cells. The X-Gal staining procedure does not permit maintenance of dendritic cell stellar morphology.

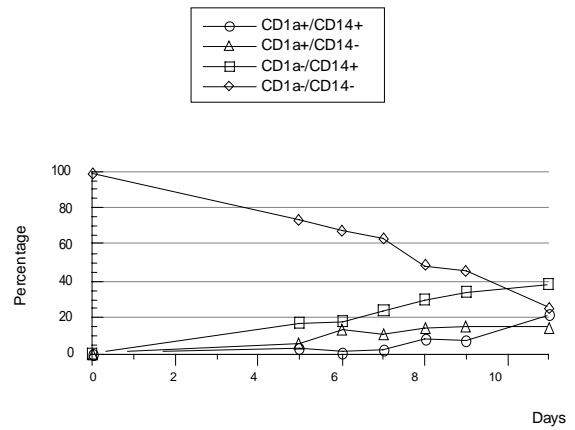


Figure 3. Percentage of CD1a⁺/CD14⁻ (triangles), CD1a⁺/CD14⁺ (circles), and CD1a⁻/CD14⁺ (boxes) and CD1a⁻/CD14⁻ (lozenges) cells in mobilized peripheral blood CD34⁺ cells-derived cell populations cultivated in the presence of GM-CSF and TNF- α . A typical experiment is shown.

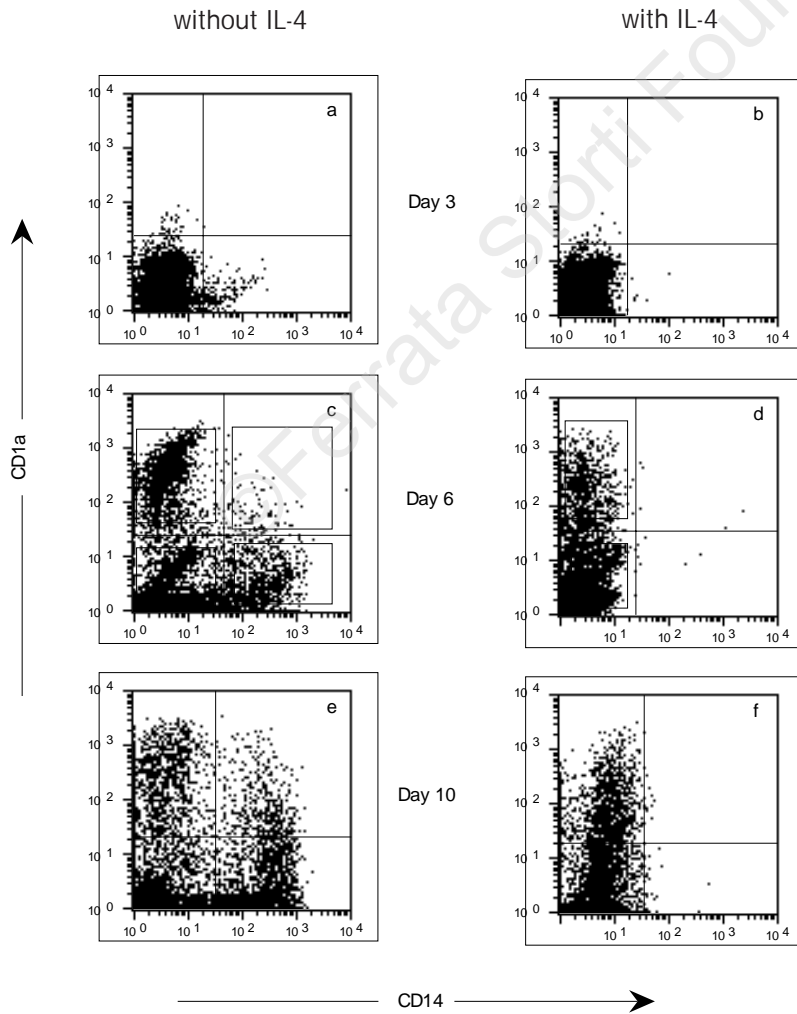


Figure 4. FACS analysis of mobilized peripheral blood CD34⁺ cells derived cell populations cultivated in the presence of GM-CSF and TNF- α at day 3 (a, b), 6 (c, d) and 10 (e, f), in the presence (a, c, e) or absence (b, d, f) of IL-4. Experiments were performed using a two-color fluorescence staining strategy with PE-conjugated anti-CD1a and FITC-conjugated anti-CD14 monoclonal antibodies. The gating strategies used to sort CD1a⁺/CD14⁻, CD1a⁺/CD14⁺, CD1a⁻/CD14⁺, and CD1a⁻/CD14⁻ cells at day 6 are depicted in panels c and d. Control experiments were performed with irrelevant mouse monoclonal antibodies.

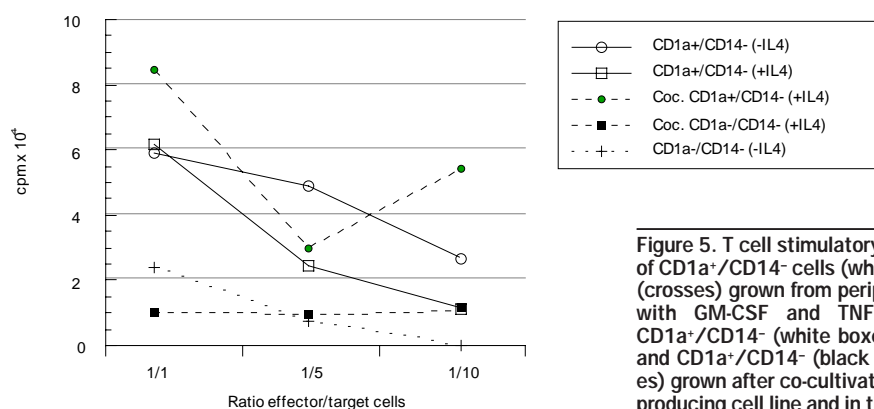


Figure 5. T cell stimulatory function (primary allogeneic MLR) of CD1a⁺/CD14⁻ cells (white circles), and CD1a⁻/CD14⁻ cells (crosses) grown from peripheral blood CD34⁺ cells cultivated with GM-CSF and TNF- α in the absence of IL-4; of CD1a⁺/CD14⁻ (white boxes) grown in the presence of IL-4; and CD1a⁺/CD14⁻ (black circles), CD1a⁻/CD14⁻ (black boxes) grown after co-cultivation (Coc.) with the retroviral vector producing cell line and in the presence of IL-4. A typical experiment is shown. Results are expressed as mean values of triplicate cultures.

U/mL IL-4 (Figure 6a). Addition of IL-4 correlated with the absence of CD1a⁻/CD14⁺ cells and the percentage of CD1a⁺/CD14⁻ was found to be markedly higher in the presence of IL-4 (Figure 6b) than in the same culture performed in the absence of this cytokine. Thus, a greater number of CD1a⁺/CD14⁻ cells was obtained after 10 days in liquid culture performed in the presence of IL-4.

CD1a⁺/CD14⁻ cells are potent stimulators in allogeneic MLR

Immunostimulation of naive T-cells was performed by mixed lymphocyte reaction (MLR). At day 6, cells were sorted on the basis of CD1a and CD14 expression as shown in Figure 4. Highly enriched populations (more than 99% purity) were obtained. As a consequence, the CD1a⁺/CD14⁻ cell population was found enriched in cells with a typical stellar dendritic cell morphology (Figure 2b) which tend to aggregate in liquid culture (Figure 2c) as already reported.²⁰ Non-genetically modified cells were then treated with mitomycin C and co-cultivated with peripheral blood T-cells. As shown in a representative experiment (Figure 5a), the CD1a⁺/CD14⁻ subset used as the stimulating cell population was clearly more efficient to immunostimulate T-cells than the CD1a⁻/CD14⁻ subset which strongly suggests that this cell compartment contains most of the dendritic cells, if not all. This result was confirmed in five experiments demonstrating that the most powerful immunostimulatory activity was found within the CD1a⁺/CD14⁻ cell population and to a lesser extent within the CD1a⁻/CD14⁻ population (data not shown). No stimulatory activity was detected when CD34⁺ cell populations, TF-1 cell line, a human erythroleukemic cell line expressing CD34, or Jurkatt cell line were used as stimulating cells (data not shown).

Mixed lymphocyte reaction performed with sorted CD1a⁺/CD14⁻ cells derived from cell cultures established in the presence of IL-4 clearly demonstrated that

the presence of IL-4 during cell expansion does not induce loss of immunostimulatory activity of CD1a⁺/CD14⁻ cells (figure 5) and that immunostimulatory features exhibited by CD1a⁺/CD14⁻ cells were identical whether the cell populations were cultivated in the presence or absence of IL-4.

Retroviral transduction of CD34⁺ cells with MFG-S-nlsLacZ and expression in functional dendritic cells

Retroviral transduction of CD34⁺ cell population was obtained by co-cultivating target cells, CD34⁺ cells activated for 24 hrs by SCF, GM-CSF, IL3, IL6, and serum supplemented medium with A7/21, a MFG-S-nlsLacZ retroviral vector producing cell line capable of transferring the nlsLacZ gene which encodes a nuclear localized β -galactosidase. TNF- α was added after the co-cultivation process since the presence of this cytokine was found to be deleterious for the producing cell line. Afterwards non adherent cells were collected and cultivated in culture medium supplemented with TNF- α , GM-CSF, SCF for 3 days, and with or without IL-4.

The possible impact of the co-cultivation step on the generation of dendritic cells was estimated by analyzing morphology, phenotype of cell populations, and immunostimulatory activity. No difference was observed whether the CD34⁺ cell populations were prestimulated and co-cultivated with A7/21 or directly induced to differentiate into dendritic cells without co-cultivation. Growth kinetics were found to be markedly increased during co-cultivation with the retroviral vector producing cell line, but remained identical to mocked-cells after the co-cultivation period (data not shown).

Transduction efficiency ranged from 8 to 55% as assessed by X-Gal staining of DCs derived from transduced CD34⁺ cell populations performed 8 days after co-cultivation (Figure 2d). When X-gal expression was measured on populations sorted on the basis of

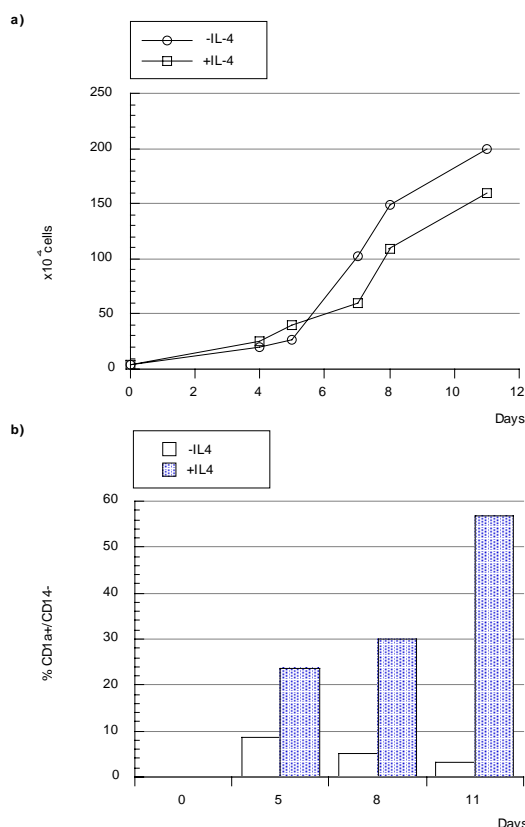


Figure 6. Comparative growth kinetics and percentage of CD1a⁺/CD14⁻ cells from peripheral blood CD34⁺ cells cultivated with GM-CSF and TNF- α in the presence or absence of IL-4.

a) Four $\times 10^4$ freeze-thawed CD34⁺ cells were seeded into complete culture medium supplemented with 10 ng/mL GM-CSF, 2.5 ng/mL TNF- α , and for the first three days with 100 ng/mL SCF (c-kit ligand), in the presence (boxes) or absence (circles) of IL-4. Both adherent cells and non-adherent cells were collected on the days indicated and counted.

b) On the days indicated, the percentage of CD1a⁺/CD14⁻ cells present in cell populations derived from peripheral blood CD34⁺ cells cultivated with GM-CSF and TNF- α in the presence (gray columns) or absence (white columns) of IL-4 (white columns), were estimated by FACS analysis (see materials and methods). A typical experiment is shown.

CD1a and/or CD14 expression, dark blue cells were found both in dendritic (3.9 to 22.5%) and macrophage (7.1 to 28.9%) cell compartments. The addition of IL-4 did not modify, *per se*, the percentage of transduced cells (data not shown), but it led to an increase in the absolute number of CD1a⁺/CD14⁻ cells which include cells expressing the nlsLacZ gene.

Discussion

A retroviral vector able to transfer and express the nlsLacZ gene, which encodes for a bacterial β -galactosidase whose activity can be histochemically detected and used as a marker, was used to study the pos-

sibility of transducing CD34⁺ progenitors purified from peripheral blood after mobilization by G-CSF prior to induction to differentiate into dendritic cells.

CD34⁺ cells induced to proliferate and differentiate by SCF, TNF- α , GM-CSF plus or minus IL-4 gave rise, after 10 days in culture, to a heterogeneous cell population which included dendritic and macrophage cells. These populations can be discriminated by phenotypic and morphological studies. According to the expression of cell surface markers three main populations were observed: CD1a⁺/CD14⁻, CD1a⁺/CD14⁺ and CD1a⁻/CD14⁺. The CD1a⁻/CD14⁺ population which expresses CD34 antigen at the beginning of the culture was found to decline during the period of culture. It is worth, however, noting that when sorted at day 6, this double negative cell population still retained the ability to generate dendritic and macrophage cells (data not shown). The presence of CD1a cell surface marker, when associated with the lack of expression of CD14, clearly defined a subset of dendritic cells and efforts were focused on the characterization of this population. These cells, when sorted after 6 days in culture were found to show dendritic cell morphology and to be the most efficient in stimulating peripheral blood T cells in primary MLR. As already reported these experiments showed a DC population can be generated from a G-CSF that mobilized peripheral CD34⁺ population.

To favor the differentiation towards the dendritic lineage, IL-4, a cytokine known to inhibit proliferation of macrophages, was added to the culture medium to avoid the expansion of CD1a⁻/CD14⁺ cells. A clear increase of the percentage of CD1a⁺/CD14⁻ dendritic cells was obtained in the presence of IL-4 which suggests that generation of CD1a⁺/CD14⁻ dendritic cells and CD1a⁻/CD14⁺ macrophages were competitive in the cell culture system used. As tested by MLR, immunostimulatory activity of CD1a⁺/CD14⁻ dendritic cells cultivated in the presence of IL-4 was found not to be modified.

Assuming the presence of the retroviral receptor on peripheral blood mobilized CD34⁺ cells,³⁷ expression of a transgene of interest in DC cells was promoted by retroviral transduction of progenitors prior to induction to differentiate into dendritic cells. Assuming the possibility of transducing very immature cells, this strategy allows long-term expression of the transgene at different levels of differentiation. Transduction of dendritic cells with retroviral vectors implies, at least, incubation of these cells with an exogenous cell supernatant and in the case of co-cultivation close contacts with producing cells which could favor modification of cell behavior. This study shows that the transduction protocol used, co-cultivation of CD34⁺ cells with A7/21, a MFG-S nlsLacZ retroviral vector producing cell line of murine origin, does not interact with the dendritic cell differentiation pathway since no modification was observed in terms of morphology, growth kinetics, expression of selected

cell surface markers or immunostimulatory activity.

In this study we demonstrate the possibility of using the nlsLacZ gene as a marker gene to tag peripheral blood CD34⁺ cell-derived DCs as has already been envisaged for other cell types.^{35,38} Expression of the nlsLacZ gene which allows cell-per-cell visual analysis of transduced cell populations can be used to track genetically modified cells and would be of great interest when combined with therapeutic genes such as MART-1 antigen whose transduction into peripheral blood CD34⁺ DC progenitors has already been investigated.³⁹ No deleterious effect was associated with the activity of the nlsLacZ gene which was found to be strongly expressed by both macrophages and dendritic cells from two days after the co-cultivation period to the end of the culture. In terms of transduction efficiency, no clear difference was found between dendritic and macrophage progenitors according to the percentage of X-Gal⁺ cells.

This study demonstrates the possibility for dendritic cells to express a transgene of interest following genetic modification of CD34⁺ progenitors derived from mobilized peripheral blood and to retain immunostimulatory capacities. Retroviral transduction might have important advantages over other methods of antigen presentation by DCs. Peptides pulsed onto DCs stay only transiently bound to MHC molecules because of dissociation and MHC turnover which impairs long-term antigen presentation. Conversely, retrovirally transduced DCs may be able to constitutively express and process transgene products which could favor long term antigen presentation and possible immune response specific for the transgene product. This effect might appear potentially interesting when long term stimulation of the immune system is investigated in diseases such as cancers.^{39,40} In contrast, however, it could induce a deleterious effect on long term expression of a transgene introduced into stem cells to correct a genetic defect. This possibility should be taken in account when gene transfer into pluripotent stem cells is performed, with the setting-up of strategies to avoid transduction of antigen presenting cells.

Gene transfer into DCs has been suggested to be associated with adenoviral vector component specific immune response.⁴¹ Minor immune reactions against viral products unable to affect the efficiency of replication incompetent retroviral vectors have been reported,⁴² but should not be amplified by the genetic modification of DCs via gene transfer into hematopoietic stem cells assuming that interaction between viral particle components and target cells occurs at the stem cell level which does not exhibit APC properties. In addition, it should be indicated that the *ex vivo* conditions used in this study are not clinically oriented, especially when considering the use of animal serum that can promote the selection of specific CD4⁺ cells recognizing calf serum determinants and use of serum-free culture strategies

should be considered in the future as already proposed.⁴³

Several reports suggest that some DCs may participate in HIV infection⁴⁴⁻⁵⁰ which raises the possibility that genetically modified DC cells may also be used in the control or the prevention of HIV infection.

Contributions and Acknowledgments

CC performed the experimental work described in this study. RG was responsible for cell sorting technical procedures. CB, PM and CC were equally involved in the conception and design of the study. CB and PM wrote the paper. Name appearance has been decided according to previous criteria.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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