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Comparison of media and serum supplementation for generation of monophosphoryl lipid A/interferon-γ–matured type I dendritic cells for immunotherapy

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Abstract

Background aims. Ex vivo–generated monocyte-derived dendritic cells (DCs) matured with monophosphoryl lipid A (MPLA) and interferon-γ (IFN-γ) can be used as cancer immunotherapy. MPLA/IFN-γ DCs induce Th1 T cell responses and have migratory capacity. Different culture regimens have been used for generation of immunotherapeutic DCs, with varying results. In the present study, culture conditions for MPLA/IFN-γ–matured type I DCs were optimized for clinical application. *Methods.* DCs were generated from monocytes in the clinical grade culture media CellGro DC, AIM V or X-VIVO 15 in the absence or presence of 2% human serum (HS) and matured with the use of MPLA/IFN-γ. DC yield and DC functionality were assessed. DC functionality was determined by means of analysis of cytokines in culture supernatant, migratory capacity, expression of co-stimulatory molecules, T cell stimulatory capacity of DCs and T helper cell (Th) polarization by the DCs. *Results.* DCs generated in the presence of 2% HS produced low amounts of pro-inflammatory cytokines and could not migrate irrespective of the medium used. In the absence of HS, MPLA/IFN-γ DCs generated in X-VIVO did not migrate either. MPLA/IFN-γ DCs generated in AIM V have slightly lower capacity to induce Th1 cells than do DCs generated in CellGro or X-VIVO. *Conclusions.* Addition of HS to different GMP culture media is detrimental for pro-inflammatory DC maturation and migration. In the absence of serum, CellGro is the most optimal medium tested for generation of migratory and Th1-inducing MPLA/IFN-γ DCs for cancer immunotherapy.

Key Words: *clinical grade dendritic cells, culture media, immunotherapy, monophosphoryl lipid A, serum*

Introduction

Monocyte-derived dendritic cells can be used as immunotherapy to treat cancer. DCs are powerful antigen-presenting cells (APCs) that are at the basis of the adaptive immune response and have the potency to break tolerance against tumor antigens. For induction of an effective T helper cell (Th)1 T cell immune response by the DCs, maturation of DCs is important; specifically, the DCs must highly upregulate co-stimulatory molecules and produce Th1-polarizing cytokines such as interleukin (IL)-12.

Toll-like receptor (TLR) ligand-containing maturation cocktails, like polyinosinic:polycytidylic acid (pI:C)-containing cocktails (1) or monocyte-conditioned medium, IL-4, granulocyte macrophage colony-stimulating factor (GM-CSF), prostaglandin $(PG)E_2$ and tumor necrosis factor (TNF)-α (2) have been shown to induce Th1 polarization in T cells. We have previously shown that the combination of the TLR4 ligand monophosphoryl lipid A (MPLA) with interferon (IFN)-γ induces mature DCs that have the capacity to migrate, produce IL-12, polarize $CD4^+$ T cells toward a Th1 phenotype and induce superior tumor antigen-specific $CD8^+$ CTL responses (3,4).

In the present study, culture conditions were optimized for generation of clinically applicable MPLA/IFN-γ–matured DCs (mDCs) because, in addition to the maturation cocktail, the culture medium used as well as addition of serum or protein supplements have been shown to affect DC maturation and phenotype (5–7). Differences in co-stimulatory molecule expression have been described when DCs were cultured in the presence of autologous

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plasma, human serum (HS), fetal calf serum (FCS), human serum albumin (HSA) or even between different batches of HSA (5). When cultured with the use of serum-free culture media, phenotypic differences were observed between DCs cultured in different media, possibly because of the presence of different protein additives in these media (5). In addition, macrophages show different effects on tumors when generated in the presence of serum compared with the absence of serum. GM-CSF–generated macrophages show better tumoricidal activity, whereas M-CSF–generated macrophages show tumor-promoting activity when cultured in presence of serum (8), which highlights the differential effects of serum on cellular function. Moreover, addition of serum does not improve yield of DCs (2), and high amounts of serum can be detrimental for yield (9).

We show that addition of HS to different Good Manufacturing Practice (GMP) culture media is detrimental for DC maturation, migration, pro-inflammatory cytokine production and Th1 induction. Culture in different media [CellGro DC (CellGro), AIM V or X-VIVO-15 (X-VIVO)] results in DCs with small differences in phenotype. X-VIVO–cultured mDCs are incapable to migrate toward chemokine (C-C motif) ligand 21 (CCL21), whereas AIM V–cultured mDCs show the poorest cytokine production and Th1 induction. MPLA/IFN-γ DCs cultured in Cellgro show the best properties for use in cancer immunotherapy.

Methods

Generation of monocyte-derived DCs

Monocytes were isolated from fresh apheresis material of healthy volunteers (Sanquin Blood Supply, Amsterdam, the Netherlands) on informed consent by use of the Elutra cell separation system (Gambro, Lakewood, CO, USA). Purity of monocytes was confirmed by flow cytometry. Monocytes were cultured at a concentration of 0.5×10^6 cells/mL in Cellgro DC (Cellgenix, Freiburg, Germany), AIM V (Gibco/Invitrogen, Breda, the Netherlands) or X-VIVO 15 (Lonza, Walkersville, MD, USA) culture medium supplemented with GM-CSF (1000 IU/mL), IL-4 (800 IU/mL) (Cellgenix), penicillin (100 U/mL) and streptomycin (100 μg/mL) (Invitrogen) in the presence or absence of 2% HS, in 3 mL in six-well cell culture plates (Nunc, Roskilde, Denmark). After 6 days of culture, immature DCs were stimulated with 2.5 μg/mL MPLA from *Salmonella minnesota re595* (*S. minnesota re595*) (Sigma-Aldrich, Steinheim, Germany) + 1000 IU/mL IFN-γ (Immukine, Boehringer Ingelheim, Alkmaar, the Netherlands) in the absence or presence of 2% HS. Two days after stimulation, mature DCs were harvested with the use of 0.25% trisodium citrate (Sanquin Reagents, Amsterdam, the Netherlands) and resuspended in Iscove's modified Dublecco's media (IMDM) (Lonza) supplemented with 10% FCS (Bodinco, Alkmaar, the Netherlands), β-mercaptoethanol (Sigma-Aldrich), transferrin (Sigma-Aldrich), penicillin (Invitrogen) (100 U/mL) and streptomycin (100 μg/mL) (Invitrogen) and used for experiments.

Flow cytometry

To analyze the expression of co-stimulatory molecules, DCs were washed with PBA (PBS supplemented with 0.5% bovine serum albumin) and incubated in the dark with 50 μL of monoclonal antibodies or the appropriate isotype control in PBA and 3 mg/mL human γ-globulin, at 4°C for 30 min. Antibodies used are fluorescein isothiocyanate (FITC)-conjugated IgG1 isotype control, phycoerythrin (PE)-conjugated IgG2a isotype control (Sanquin Reagents), APC-conjugated IgG1 isotype control, anti–human leukocyte antigen–D-related (HLA-DR)-PE, anti–CD83-APC, anti–CD40-FITC, anti–CD80-FITC and anti–CD86-APC (BD Biosciences, San Jose, CA, USA). Cells were washed twice and resuspended in PBA. 4′,6-Diamidino-2-phenylindole (DAPI) (Sigma-Aldrich) was added before analysis to stain for cell viability and to exclude dead cells from analysis. Cells were analyzed with the use of a Beckton Dickinson Fluorescence-Activated Cell Sorting (FACS) CANTO II (BD Biosciences) and analyzed with FACS DIVA software (BD Biosciences).

Cytokine production

Production of IL-12p70, IL-6 and TNF-α was determined by means of enzyme-linked immunosorbent assay (ELISA). For detection of TNF-α and IL-6, the PeliKine-compact ELISA kit (Sanquin Reagents) was used. For the detection of IL-12p70, a combination of BT-21 monoclonal antibodies (Diaclone, Besançon, France) and C8.6 (BD Biosciences) was used in an ELISA.

Migration assay

DC migration toward CCL-21 (R&D systems, Minneapolis, MN, USA) was determined with the use of 96-well transwell plates with polycarbonate filters with 5-μmol pore size (Corning Costar, New York, NY, USA). A concentration range of CCL-21 (R&D systems) in IMDM culture medium was added to the bottom chambers and 5×10^4 DCs in 80 μL IMDM culture medium were added to the upper chamber. After 3 h of incubation, migrated cells in the bottom chamber were collected and

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