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Functional differences between dendritic cells derived from CD34+ bone marrow and peripheral blood stem cells

FEDERICA SERVIDA,* DAVIDE SOLIGO,° GIORGIO LAMBERTENGHI DELILIERS°

Fondazione Matarelli; °Centro Trapianti di Midollo, Ospedale Maggiore I.R.C.C.S., Università degli Studi di Milano, Milan, Italy

ABSTRACT

Background and Objectives. **It has been previously demonstrated that dendritic cells (DCs) are characterized by an *immature* stage with high antigen internalization capacity, followed by a *mature* stage with predominantly immunostimulatory ability. The shift from the *immature* to the *mature* state can be induced *in vitro* by the addition of tumor necrosis factor-α (TNFα). The aim of our study was to investigate the maturation steps of DCs obtained from CD34+ cells from peripheral blood stem cells (PBSC) and bone marrow (BM).**

Design and Methods**. DCs were generated *in vitro* from PBSC and BM CD34+ selected cells. The endocytic activity of the cells was measured by means of dextran-FITC uptake and alloreactivity evaluated with mixed leukocyte reactions. Immunophenotypic analysis was performed by flow cytometry.**

Results**. We observed that DCs from PBSC, in contrast to the BM derived DCs, were never able to take up soluble antigens. Mixed leukocyte reactions (MLR) performed both on PBSC and BM CD34+ derived DCs showed an allo-stimulatory activity comparable to normal controls at day 10, but significantly higher at day 14 after the addition of TNFα. Immunophenotypic analysis showed typical dendritic markers in all the samples and, after treatment with TNFα, enhanced expression of co-stimulatory molecules.**

Interpretation and Conclusions**. Our data seem to indicate that, in our culture conditions, BM-derived DCs could be efficiently used for pulsing with specific peptides, while PBSC-derived DCs, being functionally *mature*, should be more suitable for gene therapy.**

Key words: CD34+ cells, dendritic cells, antigen uptake, TNFα

Correspondence: Davide Soligo, M.D., Centro Trapianti di Midollo, IRCCS, Ospedale Maggiore, 20122 Milan, Italy. Phone: international +39-02–63632671 – Fax: international +39-02-29011564 - E-mail: dsoligo@galactica.it

Dendritic cells (DCs), highly specialized antigen-presenting cells, can be generated *in vitro* from CD34+ cells derived from various sources, such as peripheral blood stem cells (PBSC) or bone marrow (BM), with multiple cytokine combinations including granulocyte-macrohage colony-stimulating factor (GM-CSF), stem cell factor (SCF), Flt-3 ligand, tumor growth factor-β (TGFβ), interleukin-4 (IL-4) and tumor necrosis factor-α (TNFα).[1-3]

DCs can also be differentiated from CD14+ blood monocytes,[4] in which different stages of maturation have been defined.[5] *Immature* DCs have a low expression of co-stimulatory molecules, but are very efficient in antigen uptake and processing. With further maturation, they lose their endocytic capacity and acquire a full repertoire of co-stimulatory antigens and allo-stimulatory activity. The shift from the *immature* to the *mature* stage of differentiation seems to be regulated by TNFα and the proteins of the TNF superfamily.[6,7] The phenotypic and functional characterization of DCs has generally been concentrated on *mature* cells with a high capacity for antigen presentation, while relatively few works are available on *immature* CD34+ derived DCs.[8] Further studies are therefore needed, considering that antigen uptake and loading are crucial steps in any *in vitro* DC manipulation.

Design and Methods

Cell sources

Four human bone marrow (BM) and 5 G-CSF mobilized PBSC samples from healthy donors were considered for this study: all specimens were provided by bone marrow transplantation donors after informed consent. Low density mononuclear cells (LD-MNC) were separated on Ficoll-Paque gradient (specific gravity 1.077 g/mL; Nycomed Pharma, Norway).

CD34+ cell isolation

The CD34+ cells were separated using the immunomagnetic Miltenyi MACS system (Miltenyi, Germany) (mean percentage of CD34+ cells 90.6±7). In particular the mean purity of the CD34+ cells from BM was 82.2±9% while that from PBSC was 99±5%.

Cultures

The CD34+ enriched cells were grown in 25 cm^2 tissue culture flasks at a concentration of 1×10^5 CD34+ cells/mL in RPMI 1640 (Bio Whittaker, USA) with 10% fetal calf serum (FCS) (Bio Whittaker), as

well as in a *serum-free* medium (X-VIVO 15, Bio Whittaker). Various cytokines were added to both media: GM-CSF (100 ng/mL) (Sandoz, Switzerland), SCF (50 ng/mL), FIt-3 ligand (100 ng/mL), TGFβ (0.5 ng/mL), IL-4 (500 U/mL) (PeproTech, UK). After 10 days of culture TNFα was added (100 U/mL) and the cultures were carried on for 4 additional days.

DCs were also generated from monocytes according to the technique described by Sallusto and Lanzavecchia:[4] briefly, plastic adherent monocytes were cultivated for 5 days with RPMI + 10% FCS to which IL-4 (500 U/mL) and GM-CSF (100 ng/mL) (PeproTech, UK) had been added.

FACS analysis

Immunophenotypic analysis was performed by flow cytometry (FACSCalibur, Becton Dickinson, USA) on days 10 and 14 of culture with the following monoclonal antibodies: HLA-DR-FITC and PE (L243, Becton Dickinson), CD1a-PE (NA1/34, Serotech, UK), CD14-FITC (Leu-M3, Becton Dickinson), CD80-PE (L307.4, Becton Dickinson), CD83 (HB15a, Coulter, USA).

Dextran-FITC uptake

The endocytic activity of the cells was measured by means of dextran-FITC uptake. Two hundred thousands cells were incubated with 1 mg/mL dextran-FITC (MW: 70,000, Sigma) in complete medium at 4°C for 1 hour (negative control) and at 37°C for 1 hour (positive control). The cells were then washed 3 times with PBS and analyzed by flow cytometry.

Mixed leukocyte reactions

To test the allogeneic stimulatory activity of *in vitro* generated DCs, mixed leukocyte reactions (MLR) were performed in 96-well microtiter plates (Costar, USA). Peripheral blood mononuclear cells (PB-MNC) from adult donors were used as responder cells at a concentration of 1×10^5 cells/well; $CD34^+$ derived DCs were pre-inactivated with 25 µg/mL mitomycin-C (Sigma) and used as stimulator cells. After 6 days of culture, cell proliferation was measured with the WST-1 assay (Boehringer Mannheim, Germany).[9] Results were evaluated with a 1420 VICTOR multilabel counter (EG&G Wallac, Finland) and expressed as the mean of 4 replicates.

Results and Discussion

DCs derived from monocytes have been extensively studied, but little attention has been given to the antigen uptake capacity of DCs generated from $CD34^+$ PBSC and BM cells. Our results indicate that, at least in our culture conditions (both with and without serum), the cells derived from PBSC were incapable of endocytosis, measured as dextran-FITC uptake, while those derived from BM samples had a significant capacity (18.3±2.7%) (Table 1A and Figure 1), although far below the percentages obtained both in resting peripheral blood monocytes (75.4±23.7%) and in DCs generated from monocytes of the same G-CSF mobilized patients (34±10%). From day 10 to 14, TNFα was added to the cultures and the cells derived from both PBSC and BM showed no further antigen uptake capacity, but both expressed co-stimulatory molecules (Table 1B). MLR performed both on PBSC and BM $CD34^+$ derived DCs showed an allo-stimulatory activity comparable to that of normal controls at day 10, but significantly higher activity at day 14 after the addition of TNFα.

Our data may have some implications which should help in the development of DC-based immunotherapy protocols. DCs derived from $CD34^+$ PBSC have a much greater capacity for expansion but a somewhat defective maturation, mainly involving the process of antigen uptake. The use of these rapidly dividing cells could be very advantageous, particularly when retroviral gene transfer over peptide pulsing is chosen,[10] as in the case of the processing of endogenous proteins presented in the context of MHC class I molecules.[11] Monocytic-derived dendritic cells, which have been the preferential source for clinical use, do not expand during culture and have a somewhat reduced antigen presenting capacity.[12] A good compromise could be to use BM-derived DCs which can expand and are able to load and present foreign antigens efficiently.

Finally, our data clearly demonstrate the superiority of FCS-based media. After ten days of culture the cell number expanded, especially in PBSC samples grown with serum (up to 23.5 fold) and the percentage of $CD1a^+$ DCs increased. The effect of FCS was even more evident after the addition of TNFα both for BM and PBSC: $CD34^+$ derived DCs showed

Table 1. Functional and phenotypic characteristics of DCs from different sources.

		$CD34^+$ bone marrow		*$CD34^+$ PBSC*	
A	*Day 10*	*X-vivo[15]*	*10% FCS*	*X-vivo[15]*	*10% FCS*
	Fold increase	2.5±1.6	3.9±0.1	4.8±3.6	23.5±12.3
	% CD1a	17.4±1.9	30.1±6.6	1.4±1.3	23.6±12
	% HLA-DR	46.6±15	64±11.2	17.9 ±14.9	76.2±16.2
	% CD80	5.4±3.2	6.6±3.8	0.7±0.5	0.1±0.2
	% DX-FITC uptake	14.5±12.1	18.3±2.7	0.6±0.8	1.5±0.9
B	*Day 14 (with TNF-α)*	*X-vivo[15]*	*10% FCS*	*X-vivo[15]*	*10% FCS*
	Fold increase	2.6±2	3.7±0.14	5.9±2.1	5.2±4.7
	% CD1a	7.2±0.8	44.4±12.3	2.6±2.5	19.7±11.6
	% HLA-DR	46.8±16.2	78±12.3	38±17.3	83±17
	% CD80	15.8±6.3	39.5±13.2	12±0.7	37±12
	% DX-FITC uptake	2±1.4	1.9±1.4	1.6±1.5	0.7±0.3

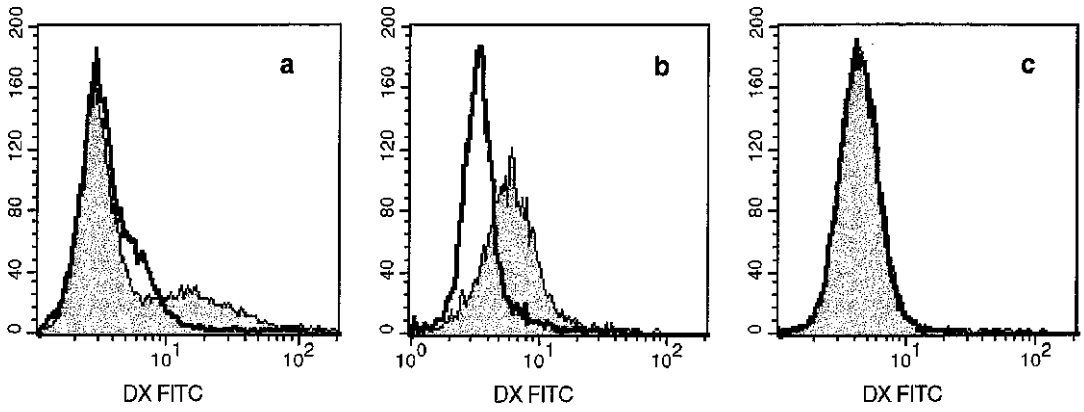

Figure 1. Flow cytometry analysis of dextran-FITC uptake (DX-FITC, SIGMA; incubated for one hour at 37°C). a): normal monocytes after five days of culture with GM-CSF and IL-4; b): CD34+ BM cells (ten days of culture with SCF, Flt-3 ligand, GM-CSF, TGFβ and IL-4;) c): CD34+ PBSC (ten days of culture with SCF, Flt-3 ligand, GM-CSF, TGFβ and IL-4). All the cultures were performed with RPMI 1640 medium additioned with 10% fetal calf serum (FCS). The open histograms are negative controls incubated at 4°C for one hour with dextran-FITC.

a *mature* phenotype, the presence of co-stimulatory molecules (Table 1B) and an increased alloreactivity in MLR. Conversely, the potential benefit of autologous plasma is still controversial.[13,14] Although highly desirable for clinical studies, the available serum-free media seem to impair full *in vitro* expansion and maturation of DCs.

Contributions and Acknowledgments

FS designed the study, performed all the in vitro assays, and wrote the paper. DS contributed to the formulation of the study, analysis of its results and writing of the paper. GLD contributed to the formulation of the design and critically revised the paper. The criterion for the order in which the names appear is based on the contribution to the work.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Potential Implications for clinical practice

- Our study shows that CD34+ PBSC derived dendritic cells have a defective maturation.
- It points out that the expansion of DCs is optimal in serum-containing media.
- It indicates that serum-free media do not allow full expression of DC antigens (for example CD80, CD1a etc).
- It could be useful for the design of dendritic cell based immunotherapy.

Manuscript processing

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