

Generation of dendritic cells from acute myeloid leukaemia cells and monocytes: our local experience

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Abstract

Dendritic cells (DC) are efficient and potent antigen-presenting cells. Pilot clinical trials indicated that DC loaded with tumour antigen could induce tumour-specific immune responses in various cancers including B-cell lymphoma, melanoma and prostate cancer. Owing to extensively low number of DC in the blood circulation, a variety of sources have been used to generate DC including monocytes, CD34+ stem cells and even with leukaemic blast cells. We demonstrate here a simple method to generate DC from acute myeloid leukaemia (AML) cells and monocytes from healthy donor or remission samples. AML cells or monocytes were cultured in RPMI 1640 media supplemented with foetal bovine serum or autologous serum where possible and different combinations of cytokines GM-CSF, IL-4 and TNF- α . The generated DC were evaluated for their morphology by phase contrast microscopy and May Grunwald Giemsa staining. Viability of cells was determined by trypan blue dye exclusion. Percentage of yields and immunophenotypes were carried out by flow cytometry. We found that cultured AML cells and monocytes developed morphological and immuno-phenotypic characteristics of DC. Monocytes are better than AML blast in generating DC and serve as a ready source for dendritic cell vaccine development.

Key words: Dendritic cells, acute myeloid leukemia, monocytes

Introduction

Dendritic cells (DC) are a unique leukocyte population, which control the primary immune response.¹ They are extremely potent antigen-presenting cells (APC), distinguished by their exceptional ability to prime naïve T cells. They lack the expression of CD3, CD14, CD16 and CD19 molecules but characteristically express high level of HLA-DR, CD1a, CD83 and costimulatory antigens such as CD80 and CD86 to promote T-cell activation. Thus, DC are central to the initiation of primary, specific immune responses and are therefore important potential vectors for the induction of anticancer immunity.² DC are derived from a common myeloid/monocytic/DC precursor and can be cultured from CD34+progenitors in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumour necrosis factor α (TNF- α)³, or from peripheral blood monocytes in the presence of GM-CSF, IL-4, and TNF- α .^{4,5} Myeloid malignancies therefore provide a unique opportunity to derive DC from the malignant cells themselves, which may then combine

expression of leukaemic antigen with the presence of the necessary costimulatory signals and be used to generate a specific antileukaemic immune response.

The major cause of treatment failure in AML is relapse of the disease. Relapse rates are considerably lower in patients who undergo allogeneic bone marrow transplantation (BMT). This is attributed to a graft-versus-leukaemia effect mediated by the donor-derived immune system, principally T cells.⁶ This effect is now being harnessed in allogeneic transplantation by means of nonmyeloablative conditioning regimens, where the conditioning and the establishment of donor-recipient chimerism have the role principally of enabling immunotherapy against leukaemic cells by infused donor lymphocyte populations. As cure rates in AML approach plateau, studies of in vivo and ex vivo immune modulation are also being extended to the setting of autologous BMT and to patients not eligible for high-dose therapies. Donor lymphocytes infusions (DLI) following allogeneic BMT are now the mainstay of treatment for early relapse of chronic myeloid

leukaemia,^{7,8} but are considerably less successful in the treatment of AML. This may be because the exponential increase in malignant cells in relapsing AML outstrips any clinical response to DLI, or AML blasts are poor antigen-presenting cells and fail to induce a sustained antileukaemic response. Additionally, the AML blasts themselves have been shown to inhibit T-cell proliferation and cytokine production through secretion of soluble factors.⁹ Certainly, in order to induce immunity rather than tolerance, the APC on which tumour antigens are presented are critical. Immunosurveillance directed against tumour antigens by host T cells may not always prevent the development of a primary malignancy, but some of these antigens may, nevertheless, subsequently function as tumour-rejection antigens if presented appropriately on DC. Data had shown that in vitro tumour-pulsed DC could provide protection against acute myeloid leukaemia in non-transplanted and transplanted mice.¹⁰

In this study, we tried to generate dendritic cells from two sources: AML blasts and monocytes from normal donor and AML remission sample. The benefits of generating DC from AML blasts include easier accessibility of AML blast and the lesser possibility of inducing autoimmunity compared to tumour lysate pulsed DC. In addition, DC are thought to principally package endogenous antigen in association with MHC class I and exogenous antigen in association with MHC class II molecules.⁹ Leukaemic DC may therefore be better able to directly prime cytotoxic T cells than DC derived from normal precursors and primed with exogenous leukaemic antigens. However, there is no proving yet for this hypothesis. In this study, morphological, cytochemical and immunofluorometric staining parameters were used to assess DC production.

MATERIALS AND METHODS

Sources of DC

Peripheral blood samples were from 2 patients, P1 and P2 with AML FAB type M4 at presentation or relapse. Samples were taken with informed consent at the time of diagnostic test or leukapheresis. To obtain homogeneous blast populations, mononuclear cells (MNC) from consecutive samples with high percentages of blast (>95%) were isolated by centrifugation of whole peripheral blood over a Ficoll-Paque density gradient (Amersham Pharmacia Biotech, Uppsala, Sweden). Mononuclear cells were also

obtained from leukapheresis of a normal donor and from remission blood sample from P2. Cells were cryopreserved in aliquots with 10% DMSO in culture medium, RPMI 1640 (Invitrogen Corporation, New York, U.S.A) containing fetal calf serum (FCS) (Life Technologies, New York, USA).

Generation of leukemic DC from AML blasts and monocytes

Cryopreserved MNC from leukaemic blasts were thawed and washed in culture medium containing 10% FCS. All the thawed samples had viability of more than 94%. Cells were suspended at 5×10^5 cells per ml culture medium with various combinations of cytokines (BioSource International, Inc., CA, USA). Samples were cultured with 1000U/ml GM-CSF, IL-4 and 100ng/ml TNF at day 0 or day 5.¹¹ All cultures were maintained at 37 °C in 5% CO₂ and a humidified atmosphere. Cytokines were topped up at day 3. For MNC obtained from normal donor or remission patient, 1×10^6 /ml cells were incubated for 2 hours and non-adherent cells were removed by sloshing the media and discarded.¹² Adherent cells were rinsed twice with culture media. The cells were cultured in a similar way as for leukaemic blasts. Cultured cells were observed by phase-contrast microscopy for evidence of increasing size and cytoplasmic projections. On day 7 to day 8, cells were harvested, and cell count and viability were assessed by trypan blue dye exclusion. Cytocentrifuge preparations of cultured cells were stained with May Grunwald Giemsa and immunocytochemical staining with alkaline phosphatase (ALP) system for CD1a and HLA-DR (Dako, CA, USA).

Immunophenotype of fresh and cultured cells by flow cytometry

Unmanipulated and cultured AML samples were stained with fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, and peridinin chlorophyll protein (PerCP)-conjugated mouse monoclonal antibodies (mAbs) against CD1a, CD14, CD86, CD80, CD83 (Pharmigen, CA, USA) and HLA-DR (Becton Dickinson, CA, USA), or with appropriate isotype control mAbs. Cells were incubated with mAbs for 15 minutes in room temperature, washed once in phosphate buffered saline, and resuspended in 1ml PBS for analysis by means of a FACScan flow cytometer (Becton Dickinson, CA, USA) with CellQuest (Becton Dickinson, CA, USA) software. Forward- and side-scatter gates were established to exclude

cell debris and clumps prior to analysis for expression of each phenotypic marker.

RESULTS

Cultures

After 7 to 8 days of culture, cells had increased in size, acquired copious gray cytoplasm and

multiple projections (Figures 1 & 2). Cells also stained positive for CD1a and HLA-DR with ALP system (Figure 3). Cells number and viability were assessed by trypan blue dye and haemocytometer. Cells culture results were summarized in Table 1. Percentage recovery of cultured cells was calculated by dividing the

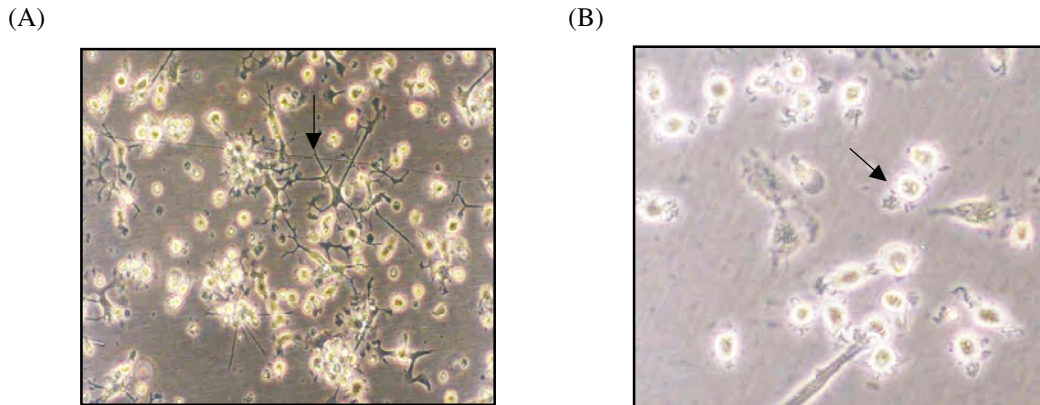


FIG. 1: Phase contrast microscopy of generated DC from AML blasts (A) and monocytes (B). Arrows show typical multiple projections of dendritic cells. Magnification X 200.

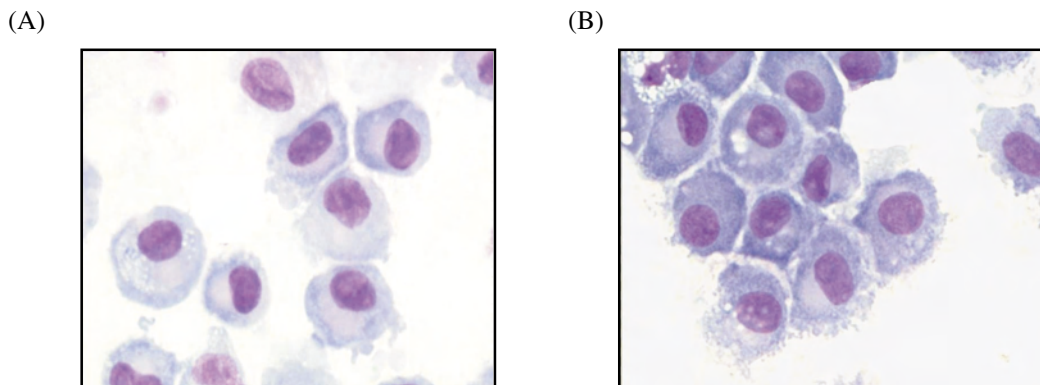


FIG. 2: Cultured AML blasts (A) and monocytes (B) stained with MGG. Magnification X 400.

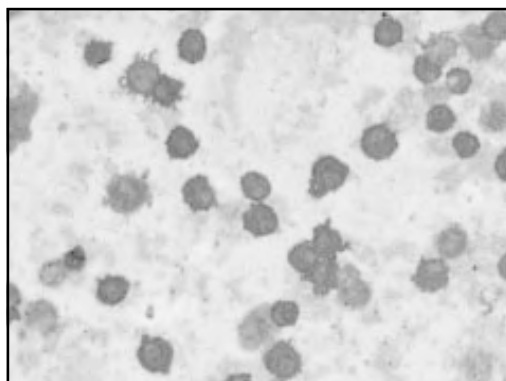


FIG. 3: Cultured monocytes stained with CD1a via ALP system. Magnification X 100.

total cells number harvested at the end of culture by initial plated cells number, and then multiplied by 100%. The mean percentage of recovery for cultured normal MNC was $9\pm 4\%$ whereas for cultured AML blasts was $80\pm 51\%$. The mean viability of cultured monocytes was $86\pm 2\%$ and for cultured AML blasts was $66\pm 16\%$. As cultured cells longer than 9 days had low viability, our samples were cultured for 7 to 8 days. We found that the duration were sufficient to generate mature dendritic cell. We also found that addition of $TNF-\alpha$ at Day 0 to monocytes produced more adherent cells that developed into macrophages.

Immunophenotype

The immunophenotype of the cultured blasts and monocytes was compared with the uncultured blast or monocytes (Figure 4). Cells that combined high-level expression of MHC class II and expression of at least one of CD86, CD80, CD83 and CD1a with very low-level expression of CD 14 were defined as DC immunophenotypically. Mean expression of CD1a+/HLA-DR+ dendritic cells was $90\pm 2\%$ for cultured monocytes and $15\pm 9\%$ for cultured AML blasts. The percentages of expression of each of the phenotypic markers of DC were also listed in Table 1. Cultured monocytes had higher

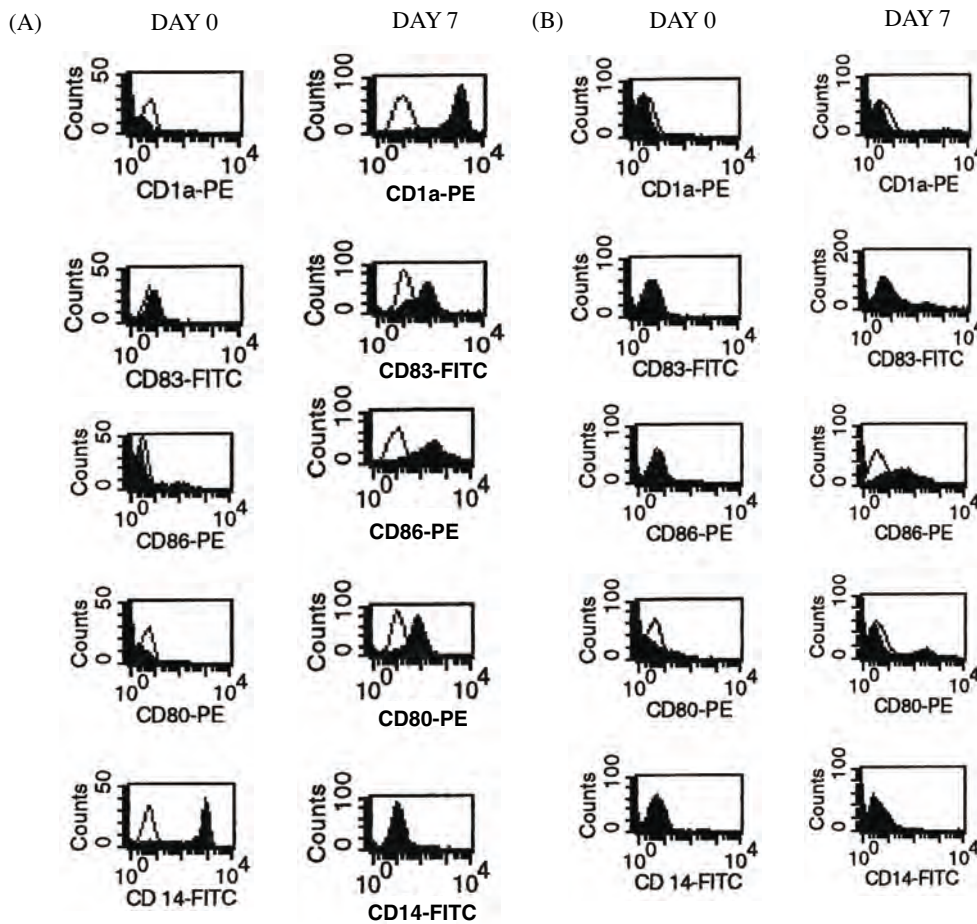


FIG. 4: Histogram plots of FACS analysis for : (A) monocytes and (B) AML blast from P1 at day 0 and day 7. Dotted line depicts isotype control.

TABLE 1: Culture data and percentage expression of surface markers.

	Cultured monocytes	Cultured remission monocytes from P2	Cultured blast from P1	Cultured blast from P2
% Viability on the day of harvest	84	87	81	50
% of recovery from plated MNC	12	5	130	29
% of CD1a+/HLA-DR+ cells	92	88	23	6
% of CD80+ /HLADR+ cells	84	85	26	11
% of CD86+/HLA-DR+ cells	84	75	30	n.a.
% of CD83+/HLA-DR+ cells	71	70	12	3
% of CD14+ cells	2	n.a.	3	0

n.a.: data not available

expression of all the DC markers compared to cultured AML blasts.

DISCUSSION

In this study, we have successfully generated leukaemic DC from 2 AML patients. The generated DC not only morphologically appear to look like DC but also express surface antigens typical of DC such as CD1a, CD83, CD80, CD86 and HLA-DR. However, the percentages of viability and recovery of the cultured cells varied. P1 depicted a better viability and percentage of recovery i.e. 81% and 130% compared to P2, 50% of viability and 25% of recovery. This means that blasts from P1 grew better and contributed to more than 100% of recovery. This is true when observed under the microscope; blasts divided and formed colonies during the culture. For P2, no colony was observed. Our finding was similar to those reported by other groups^{11,13} which had shown that different samples had different capability to survive and mature towards DC despite of being the same subtypes. From our culture of P1 blasts, we found that cells cultured more than 7 days would produce more adherent cells with longer dendrites. When phenotyped, these cells also expressed markers of DC. This observation

was not reported before. We believed that more matured DC would adhere to the flask. Therefore, although DC was reported non-adherent in culture, our observation suggests that DC can adhere to the flask on further culture.

Until today, many groups have tried to generate DC from leukaemic blast cells with different concentration and combination of cytokines and media. However, spontaneous differentiation of a proportion of cells from Juvenile myelo-monocytic leukemia (JMML) had been reported.¹⁴ Further study on whether a spontaneous differentiation of AML blasts to DC needs to be carried out in order to clarify this issue. Besides that, the functional properties of these DC whether they suppress or elicit protective immune response in this disease need to be determined too.

We had shown that with our culture conditions, monocytes either from normal donor or remission samples would be able to differentiate into DC. Both samples had good viability and yield. In our observation, on day 3, all the adherent monocytes from remission sample become non-adherent and form colonies. However, from our experiments, if TNF- α were added at day 0 of culture, no colony was observed and many macrophage-like adherent cells were formed.

In conclusion, we have shown that DC can be generated from AML blasts and monocytes under our modified culture conditions. The yield of DC is much better from AML blasts compared to monocytes. These DC can be used for further experimental study such as mixed leucocytes reaction to evaluate auto- or allo-stimulatory activity of T cells. Subsequently, these DC can be used as a source of cellular vaccine for elimination of residual disease or protection of relapse, or even therapeutic intervention of early relapse.

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