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ORIGINAL ARTICLE

Observation of dendritic cell morphology under light, phase-contrast or confocal laser scanning microscopy

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Abstract

Dendritic cells (DCs) are professional antigen presenting cells of the immune system. They can be generated *in vitro* from peripheral blood monocytes supplemented with GM-CSF, IL-4 and TNF alpha. During induction, DCs will increase in size and acquire multiple cytoplasmic projections when compared to their precursor cells such as monocytes or haematopoietic stem cells which are usually round or spherical. Morphology of DCs can be visualized by conventional light microscopy after staining or phase-contrast inverted microscopy or confocal laser scanning microscopy. In this report, we described the morphological appearances of DCs captured using the above-mentioned techniques. We found that confocal laser scanning microscopy yielded DCs images with greater details but the operating cost for such a technique is high. On the other hand, the images obtained through light microscopy after appropriate staining or phase contrast microscopy were acceptable for identification purpose. Besides, these equipments are readily available in most laboratories and the cost of operation is affordable. Nevertheless, morphological identification is just one of the methods to characterise DCs. Other methods such as phenotypic expression markers and mixed leukocyte reactions are additional tools used in the characterisation of DCs.

Keywords: Dendritic cells, inverted phase contrast microscope, light microscope, confocal laser scanning microscope.

INTRODUCTION

Dendritic cells (DCs) constitute a system of cells crucial to the immune response, especially the T-cell mediated immunity. DCs exist in a number of places in the body and are mobile, wandering in the blood and lymph from peripheral organs to the lymphoid organs, especially to T-cell areas such as that in the lymph nodes.[1] Their involvement in antigen presentation to the immune system plays a vital role in the induction of anti-tumour responses.[2]

DCs can be directly isolated from blood; or generated *in vitro* from peripheral blood monocytes or CD34+ bone marrow cells. In the presence of granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4, immature DCs are generated. The maturation process is promoted by incubation with tumor necrosis factor (TNF)-α, stem cell factor, or FLT3 ligand.[3-6] Upon maturation, DCs will increase in size and develop extensive cytoplasmic projections in many directions from the cell body. DCs can be further characterised by immunophenotyping using flow cytometry. Mature DCs lack cell surface antigens in association with hematopoietic lineages such as CD19, CD20 for B cells, CD3 for T cells, CD56 for NK cells, CD14 for monocytes and CD34 for haemopoietic stem cells. DCs express high level of MHC class I and co-stimulatory molecules such as CD80 and CD86[5] which facilitate both the interaction with and stimulation of lymphocytes.[7]

During cell culture, morphological characteristics of DCs can be observed using live cell imaging system for evidence of their increased size and cytoplasmic projections on the cells. Several other methods can be utilized to observe and capture the microscopic image

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of the cells. These include light microscopy by fixing and staining the cells, phase-contrast inverted microscopy with or without colorimetric or fluorescence cytochemical cells labeling, confocal microscopy and electron microscopy.

In this paper, we reported the morphological images of mature DCs using three different methods of sample processing and observing under light microscopy, phase contrast inverted microscopy and confocal laser scanning microscopy. Their identity was confirmed by immunophenotypic analysis using flow cytometry.

MATERIALS AND METHODS

Patients and sample collection

Five healthy volunteer blood donors were recruited in this study with informed consent. 20 mL of blood was drawn into vacutainer tubes (Becton Dickinson Vacutainer; Beckton Dickinson, UK) containing EDTA. Blood samples were used for blood monocyte isolation. Unwanted components were discarded following standard procedures of the laboratory.

Myeloid-derived DCs generation

Dendritic cells were generated from peripheral blood monocytes and maintained in culture as described previously[8-9]. Briefly, blood was diluted with phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} (Miltenyi Biotec, Germany).in 1:1 ratio and layered on Ficoll-paque Plus (GE Healthcare, UK) for density-gradient centrifugation. The ratio of blood to ficoll-paque was as recommended by the manufacture. Mononuclear Cells (MNC) were isolated from the interface. The obtained MNC were washed with two parts of PBS.

The isolated MNC were then subjected to monocyte isolation using CD14 magnetic microbead positive selection. CD14 is a 55 kDa leucine-rich repeat protein expressed strongly on the surface of monocytes and other myeloid cells.[10-13] The obtained monocytes were then seeded on T25 culture flask (Nunclon™, Nunc, Denmark) at a cell concentration of 2 x 10^6 cells/ml in Rose Roswell Park Memorial Institute (RPMI 1640) (Gibco, Carlsbad, USA) culture medium supplemented with cytokines including 1000 U/ml of GM-CSF and IL-4 (Chemicon, USA), 10% fetal bovine serum and 1% v/v penicillin and streptomycin (Gibco, Carlsbad, USA). Half medium exchange was performed every other day with fresh cytokine-supplemented medium for a total of 7 days. 100ng/ml of TNF-alpha (Chemicon, USA) was added to the above-mentioned culture medium to promote maturation of DCs from day 7 to the day 14. During this period, the culture cell were observed throughout the culture period and at the end of the culture by inverted phase-contrast microscope (Carl Zeiss Axiovert 200) for evidence of increasing in size and cytoplasmic projections on the cells. The generated DCs were of myeloid-derived DCs and their identity was confirmed by immunophenotypic analysis using flow cytometry.

Microscopic observation of DCs

At the 14th day of the culture, cells were harvested for morphology observation using three different methods:

Light microscopy of MGG-stained DCs: For May Grunwald Giemsa (MGG) staining, the generated DC were harvested and cytospun onto slides at 20,000 to 30,000 cells per slide at room temperature. Slides were fixed with absolute methanol for 15mins. After fixation, the slides were air-dried and diluted May Grunwald reagent (BDH Laboratory, UK) was applied on to the slides for five minutes. Excess of May Grunwald reagent was removed prior to the addition of diluted Giemsa reagent. Cells were incubated with Giemsa reagent for 20 minutes at room temperature. After incubation, slides were washed with running tap and dried. Dried slides were mounted in dibutyl phthalate xylene (DPX) (BDH Laboratory, UK) with a cover slip. Microscopic images of the stained DC were observed and captured with Carl Zeiss Axiovert 200 microscope.

Phase-contrast inverted microscopy: At the end of the culture, DCs were observed from the culture flask under phase-contrast inverted microscope. Images of DCs were captured using a Carl Zeiss Axiovert 200 microscope. No specific staining was carried out.

Confocal Laser Scanning Microscopy: For this technique, 500μL of the DCs cell culture was withdrawn and put on glass slide with a cover slip without staining. The cells were observed and their images were captured using a Carl Zeiss LSM 700 Laser Scanning Microscope and Zen software.

RESULTS

The cultured cells were observed using inverted phase-contrast microscope throughout the culture period as mentioned. When monocytes were seeded at the beginning of the culture, they were spherical and adherent to the plastic surface of the culture flask. At the third day of the culture, cells were found to be irregular in shape and semi-detached from the plastic surface of the culture flask. At day five, these cultured cells were found clumping up forming colonies suspending in the culture medium. Upon stimulation of maturation by TNF-alpha, cells were transformed into single cell suspension with cytoplasmic projections (dendrites). DCs were generated from their precursor cells, monocytes, and their identity was confirmed by immunophenotypic analysis by flow cytometry. At the end of the culture, the generated DCs showed stellate processes giving veiled appearance which are consistent with the morphological description of dendritic cells.[5, 14]

Under the phase contrast microscope, Figure 1 and 2 showed the irregular shape of the cultured cells with cytoplasmic projections. May-Grunwald Giemsa stain (Figure 3) and confocal laser scanning microscope (Figure 4) provided better resolution of the cell image. Under confocal microscopy, these cultured cells were found not only with irregular shape but also abundant phase-dense granules and irregular nucleus with small nucleoli. The image captured using confocal microscope showed more extended dendrites projecting in many directions from the cell body (Figure 4) than that captured by inverted microscopy or light microscopy.

DISCUSSION

DCs derived from myeloid lineage are potent antigen-presenting cells (APC) responsible for anti-tumour immunoreactivity.[5,14] The distinctive dendrite formation of DCs serves as a vital feature for morphological identification of DCs generated in-vitro. In this study, we have generated DCs from monocytes, and captured the microscopic images of these cells using three different techniques.

When studied under the inverted phase contrast microscope, DCs were observed in suspension. DCs were usually seen overlapping one another giving poor resolution images as shown in Figure 1 and 2. Nevertheless, this type of microscopy allows live cell imaging without requiring staining the cells. It also allows the flexibility of the time to observe the cells throughout the culture period.

DCs could also be observed under the light microscope after staining. In this technique, DC suspension was cytospun and plated as a thin monolayer of cells onto a glass slide prior to fixing and staining with MGG. However, the cytospinning process is damaging to the cell,[12] hence the images of the MGG stained

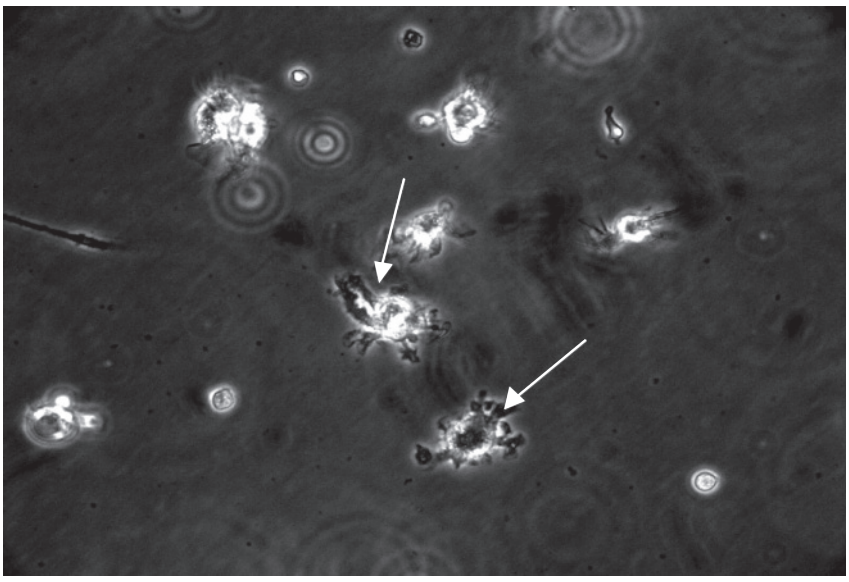


FIG. 1: Photomicrographs in black and white showing morphology of dendritic cells (DCs) derived from monocytes. Typical DC morphology (see white arrows) with dendrites was observed (200 x magnification).

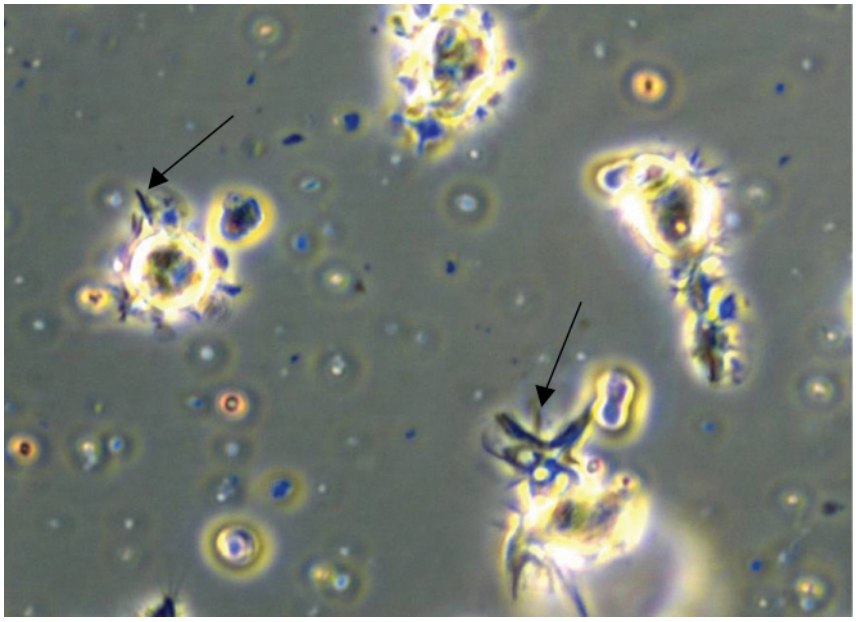


FIG. 2: Live morphological observation of mature DCs in phase contrast at 400x magnification. Cells are of irregular shape showing dendrites formation giving veiled appearance (see black arrows).

DCs may be compromised. The MGG stain is a Romanowsky stain commonly used for staining peripheral blood and bone marrow smears. The staining reaction is based on electrostatic interaction between dye and target molecules. The basic methylene blue azure compliments of the stain precipitate at acidic components of the cellular constituents such as the cytoplasm and chromatin.[15] DCs stained with MGG were as illustrated in Figure 3. The spinning and staining processes could have damaged the dendrites and hence the irregular dendrite formation and fewer number of dendrites. This method, however, allows preserving of the cell morphology on the slides and retrieval of stored slides for later use.

Confocal laser scanning microscopy enables in-focus image acquisition from selected depths, known as optical sectioning. Images could be acquired point-by-point and reconstructed with a computer, allowing three-dimensional reconstructions of topologically-complex objects.

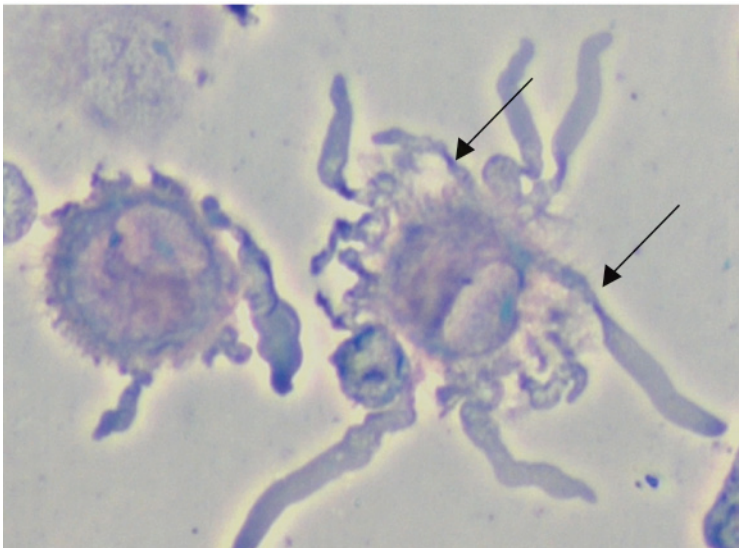
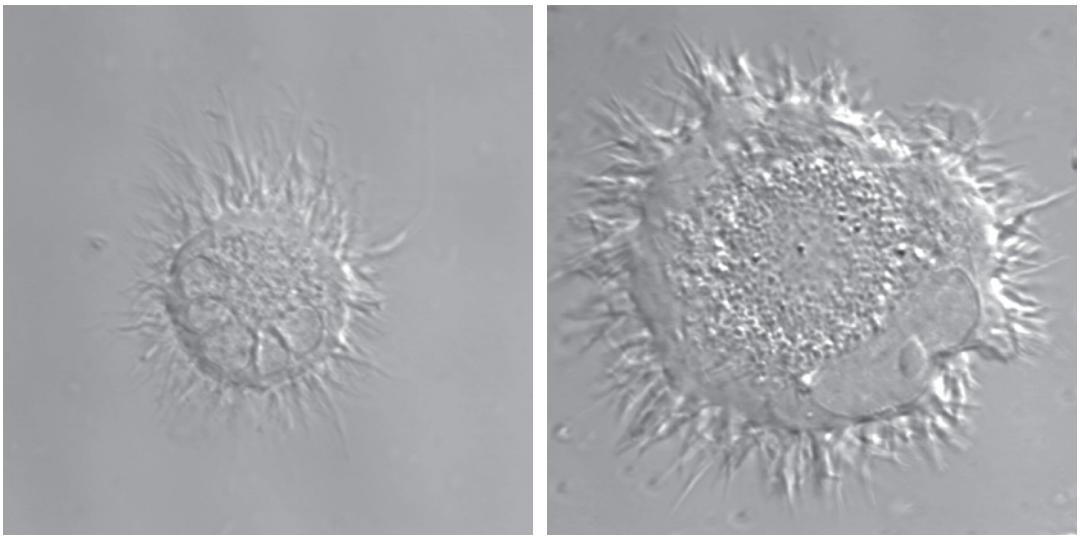


FIG. 3: Photomicrograph of DCs stained with May-Grunwald Giemsa (MGG). DCs were found displaying typical morphology with long cytoplasmic projections (see black arrows) at magnification 400x.



(a) 37x (b) 37x

FIG. 4: Live morphological observation of DCs of different sizes on glass slide under confocal laser scanning microscopy at (a) and (b) brightfield 37x magnification showing numerous extended dendrites in many directions from the cell body with abundant phase-dense granules and irregular nucleus with small nucleoli.

Thus, it is useful not only for cell surface profiling, but also for assessing the interior structures of the cells.[16-18] In this study, cell surface of the cultured DCs was analysed with confocal laser scanning microscope. A drop of medium containing the DCs were withdrawn from the culture flask and placed on a glass slide and covered with a slip. Profusion of dendrites on the DCs was clearly illustrated in the captured images. This microscopic analysis offers high resolution images and simple cell preparation under bright field observation. The DC cytoplasmic and nuclear appearances are better appreciated with this technique.

Some morphological variations especially cytoplasmic projections of DCs were observed under these three different microscopy techniques. During the observation of live DCs in cell culture medium under the inverted phase contrast microscope, we merely captured single plane image of a three-dimensional cell in suspension. Hence, fewer dendrites of the cells were observed. On the other hand, when DCs were cytospun (for light microscopy) or layered (for confocal laser scanning microscopy) onto glass slides and were compressed by a cover slip to a single plane dimension, more dendrites were observed. As mentioned earlier, cytospinning is a damaging process to the cells, hence, MGG stained DCs were observed with fewer dendrites under the light microscope as compared with non-cytospun cells observed under the confocal laser scanning microscope. Therefore, the images captured with confocal laser scanning microscopy were more realistic.

The light microscope and inverted phase contrast microscope are readily available in most laboratories as the basic equipments for cell culture. Therefore, it is convenient and cost saving to utilize ready equipments for observation of cell morphology. Besides, MGG stain is a common economical chemical having long shelf life and stability. On the other hand, the confocal laser scanning microscope offers high resolution of cell images, but it is expensive and requires an experienced operator to capture and analyze the image with optimal outcome.

In conclusion, DCs generated from monocyte can be assessed by various techniques in which confocal laser scanning microscope offers the most realistic image when compared to light microscopy after staining and inverted phase contrast microscopy.

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REFERENCES

1. Nairn R, Helbert M. Immunology for Medical Students, London: Mosby International Ltd 2002.

2. de Vries IJ, Eggert AA, Scharenborg NM, et al. Phenotypical and functional characterization of clinical grade dendritic cells. J Immunother. 2002; 25(5):429-38.
3. Steinman RM. The dendritic cell system and its role in immunogenecity. Ann Rev Immunol 1991; 9:271-96.
4. Levin D, Constant S, Pasqualini T, Flavell R, Bottomly K. Role of dendritic cells in the priming of CD4+ T lymphocytes to peptide antigen in vivo. J. Immunol 1993; 151:6742-50.
5. Hart DNJ. Dendritic cells: unique leukocyte populations which control the primary immune response. Blood 1997; 90:3245-87.
6. Mothy M, Jourdan E, Mami NB, Vey N, Damaj G *et al*. Imatinib and plasmacytoid dendritic cell function in patients with chronic myeloid leukemia. Blood 2004; 103: 4666-8.
7. Stockwin LH, McGonagle D, Martin IG, Blair GE. Dendritic cells: immunological sentinels with a central role in health and disease. Immunol Cell Biol 2000; 78: 91
8. Lim MN, Leong CF, Cheong SK. Generation of dendritic cells from acute myeloid leukaemia cells and monocytes: Our local experience. Malays J Pathol 2003; 25: 107-12.
9. Tan YF, Sim GC, Habsah A, Leong CF, Cheong SK. Experimental production of clinical-grade dendritic cell vaccine for acute myeloid leukemia. Malays J Pathol 2008; 30(2): 73-9.
10. Ferrero E, Hsieh CL, Francke U, Goyert SM. CD14 is a member of the family of leucine-rich proteins and is encoded by a gene syntenic with multiple receptor genes. J Immunol. 1990;145:331-6.
11. Simmons DL, Tan S, Tenen DG, Nicholson-Weller A, Seed B. Monocyte antigen CD14 is a phospholipid anchored membrane protein. Blood 1989; 73: 284–9.
12. Kim J-I, Lee CJ, Jin MS, Lee C-H, Paik S-G *et al*. Crystal structure of CD14 and its implications for lipopolysaccaride signalling. J Immunol. 2005; 280: 11347–51.
13. Landmann R, Link S, Sansano S, Rajacic Z, Zimmerli W. Soluble CD14 activates monocytic cells independently of lipopolysaccharide. Infect Immun. 1998; 66: 2264–71.
14. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998; 392: 245-52.
15. Bain BJ. Blood cells: A practical Guide. 3rd ed., London: Black Well Publishing 2002.
16. Papworth GD, Stolz DB, Watkins SC. Imaging dendritic cells: A primer. In: Lotze MT, Thomson AW. Dendritic cells: Biology and clinical applications, Academic Press. London. 2001: 231
17. Cox, G. Biological confocal microscopy. Materials Today 2002; 5: 34-41
18. Wright SJ, Wright DJ. Introduction to confocal microscopy. Methods in Cell Biology 2002; 70: 1-85.