PHCbi

Application Note

LiCellMo™

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Metabolic shift monitoring of immune cells during differentiation

Introduction

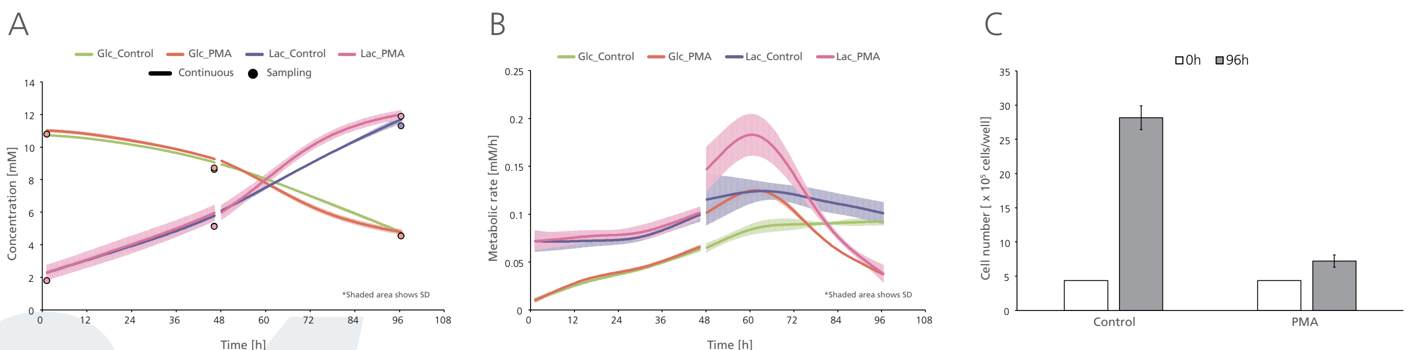
Monocytes and macrophages play an essential role in the body's natural immune system. Monocytes are produced in the bone marrow and move around the body in the blood, differentiating into macrophages and infiltrating tissues. Activated macrophages become dependent on the glycolytic pathway for energy production, even in the presence of oxygen; this metabolic profile, called the Warburg effect, is a well-known characteristic of cancer cells.[1] The Warburg effect has been widely studied in immune cells, but it is reported to serve different purposes in cancer cells and in immune cells. In cancer cells, the Warburg effect is for survival and proliferation, whereas in immune cells, it is a sign of aging and cell death.[2,-4] In monocytes and other immune cells in which the Warburg effect may be observed, the metabolic profile differs for each state of immune activity. For example, monocytes and M2 macrophages show increased fatty acid oxidation and oxidative phosphorylation, whereas M0 and M1 macrophages show increased glycolysis. In addition, the microenvironment and metabolic profile of M0 macrophages are known to be involved in controlling polarization of M1 and M2 macrophages.[5] This means that investigating the metabolic profile of immune cells can allow inferences to be made about their differentiation state.[6,7] In this study, chronological monitoring of metabolic shifts in THP-1 cells, a monocyte cell line, when they differentiated into M0 macrophages as a result of exposure to PMA, was carried out.

Materials and Methods

THP-1 cells (JCRB0112.1, obtained from the Japanese Collection of Research Bioresources Cell Bank: JCRB), a cell line for acute monocytic leukemia, were used. Culture was carried out in RPMI 1640 medium with added 10% FBS for 96 hours, during which glucose and lactate concentrations in the culture medium were continuously measured using a live cell metabolic analyzer. Continuous measurement commenced from immediately after the cells were seeded, and PMA (Phorbol 12-myristate 13-acetate) was added to a final concentration of 10 ng/mL (0.001% DMSO) 48 h after seeding. In the control group, vehicle alone (0.001% DMSO) was added. The culture medium was collected for measurement of glucose and lactate concentrations when the reagent was added and at the end of culture. In addition, cell morphology was examined with a phase contrast microscope, and the expression level of CD11b on the cell surface was measured using flow cytometry.

Results

First, the accuracy of the measured values from the live cell metabolic analyzer was checked by comparing the analyzer values for glucose and lactate concentrations in the culture medium at the time of addition of the reagent and at the end of culture against values from the colorimetric method. The maximum deviation was 14%, and it was therefore concluded that the measured values from the live cell metabolic analyzer were sufficiently accurate (Fig. A). Following this, the glucose consumption rate and the lactate production rate were calculated from the continuous changes in glucose and lactate concentrations (Fig. B). Comparison of these values showed that the glucose consumption rate and lactate production rate increased immediately after the addition of PMA. The cell number at the end of culture was higher in the control group, suggesting that per cell glucose consumption and lactate production increased as a result of addition of PMA (Fig. C). Furthermore, the microscopic images confirmed that the monocytes, which are non-adherent cells, changed into adherent cells as a result of the addition of PMA (Fig. D). The level of expression of CD11b, a marker for macrophages, on the surface of the cells was measured by flow cytometry, and the results showed that the level of expression of CD11b increased as a result of the addition of PMA (Fig. E). These results confirm that the monocytes differentiated into macrophages due to the addition of PMA.



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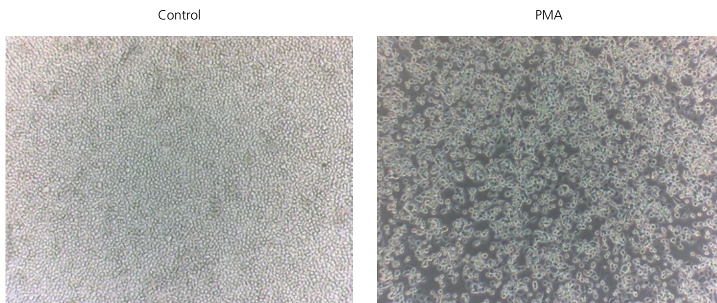

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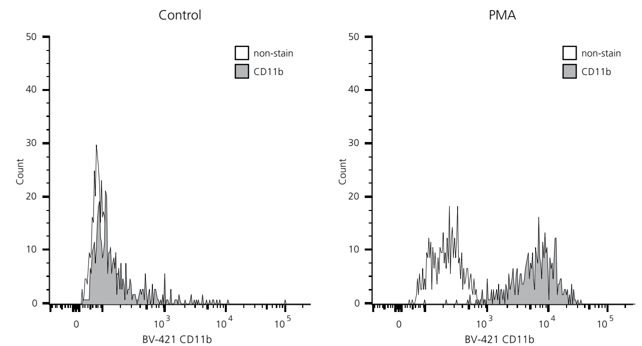

Discussion

By comparing metabolic rates analyzed by a live cell metabolic analyzer and cell numbers, it was possible to demonstrate that monocytes, which have increased fatty acid oxidation and oxidative phosphorylation, show an enhanced glycolytic pathway as they differentiate into macrophages. However, at 60 h of culture, 12 h following addition of PMA, the metabolic rate of macrophages began to drop. Suppression of glycolysis and reduction of immune cell function occur in response to the lactate concentration of cellular environment, and this is reported to be one of the reasons for the lack of immune response in the tumor microenvironment.[8] In addition, M1 macrophages cultured in medium with 5–20 mM lactate are reported to have a tendency toward reduced glucose uptake.[2] In the present experiment, the lactate concentration had reached approximately 8 mM at the point at which the metabolic rate began to decrease, suggesting that suppression of glycolysis due to the increased lactate concentration in the medium was reflected in the reduction in metabolic rate.

Conclusion

By using a live cell metabolic analyzer, chronological measurement of the metabolic shift during the process of differentiation of monocytes into macrophages was performed. The metabolic profile of immune cells is intimately related to their differentiation state, and observation of the metabolic profile of immune cells in different states of differentiation, as in the present experiment, will contribute to the evaluation of immune cell differentiation. The live cell metabolic analyzer measures the concentrations of glucose and lactate while the cells are being cultured, thus allowing chronological observation of the metabolic shift during the process of cell differentiation. Use of this technique in studies in which immune cells are made to differentiate into a wide variety of phenotypes may be expected to bring new insights in metabolic research.

Reference

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*Data is the result of PHC verification and is not a guarantee of customer data.

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Version: 291124