HLA-class II antibody enhances cytokine production in human dendritic cells (Mylc-DCs) established by iPS cell technology

Ikewaki Nobunao Abraham Samuel JK*

Abstract

This study examines cytokine production as part of an immune response in dendritic cells (Mylc-DCs) expressing human leukocyte antigen (HLA)-class II (HLA-DR+DQ+DP) molecules generated by induced pluripotent stem cell technology. The Mylc-DCs were treated with HLA-class II monoclonal antibody (mAb: clone TDR31.1, mouse immunoglobulin G1) as an agonist. The Mylc-DCs showed strongly enhanced production of interleukin (IL)-6 and IL-8. The increase in their levels was observed in the culture supernatants following treatment with HLA-class II mAb but not after treatment with isotype-matched normal mouse immunoglobulin G1 antibody. Enhanced IL-6 and IL-8 production were significantly suppressed by the conventional protein kinase C inhibitor Go6976 at an optimal concentration. Overall, these findings indicate that the intracellular mechanism underlying the enhanced IL-6 and IL-8 production in the Mylc-DCs in response to HLA-class II mAb treatment (as an agonist) was closely associated with protein phosphorylation by protein kinase C activation.

Key words : cytokine, human leukocyte antigen (HLA)-class II antibody, Mylc-DCs, protein kinase C

Introduction

Dendritic cells (DCs), derived from hematopoietic bone marrow progenitor cells, are characterized by high endocytic/phagocytic activity, but low T-cell activation potential. DCs are constantly exposed to various foreign antigens in their environment, and upon contact with these antigens, become activated into DCs capable of generating and expressing peptide-major histocompatibility complex antigen-class II (MHC-class II) molecule complexes (human leukocyte antigen (HLA)-class II DR+DQ+DP) which are transmembrane proteins consisting of an α-chain and β-chain, on their cell surface, thereby acting as antigen-presenting cells (APCs), to activate $CD4^+$ T-cells and $CD8^+$ T-cells. Therefore, DCs are very important functional cells in the immune system, involved in maintaining homeostasis in the living body [1-3].

In other words, DCs take up pathogens, bacteria and viruses that invade the body and present the foreign peptides to $CD4^+$ T-cells, which are the major controller of immune responses, to induce specific immune responses against these pathogens. In addition, it is thought that DCs also present tumor-specific antigens (TSAs) expressed on tumor cells *in vivo* to $CD4^+$ T-cells, inducing an immune response against the tumor cells, contributing to their elimination from the body. Thus, DCs act as messengers between the innate and acquired immune systems to maintain humans in a healthy state [4,5].

Thus, activated DCs play important functional roles in humans, such as serving as APCs via HLA-class II molecules, and are a major controller of the innate and acquired immune systems. However, the mechanism(s) of induction of immune responses, such as cytokine production, by signal transduction via the HLA-class II molecules expressed on activated DCs is not yet clearly understood.

Department of Medical Life Science, Kyushu University of Health and Welfare School of Medical Life Science, Institute of Immunology, Junsei Educational Institution, 1714-1 Yoshino-cho, Nobeoka-shi, Miyazaki 882-8508, Japan
*Centre for Advancing Clinical Research, University of Yamanashi School of Medicine, 1110 Shimokato, Chuo-shi, Yamanashi 409-3898, Japan

We used DCs (Mylc-DCs) expressing HLA-class II molecules established by the iPS cell technology (so-called regenerative medicine technology) as a model of activated DCs to analyze the direct signal transduction through the HLA-class II pathway, because Mylc-DCs can be stably produced in large amounts and exhibit very high sensitivity to various kinds of substances [6].

In this study, we examined cytokine production, as part of an immune response, induced by HLA-class II monoclonal antibody (mAb: mouse IgG1, a monomorphic HLA-class II β-chain epitope of HLA-DR+DQ+DP) as an agonist in the Mylc-DCs, and discuss the mechanism(s) of direct intracellular signal transduction via the HLA-class II pathway from the viewpoint of protein phosphorylation by protein kinase (PK) activation.

Materials and Methods

Reagents

The conventional protein kinase C (PKC) inhibitor Go6976 was purchased from Funakoshi Co. (Tokyo). HLA-class II monoclonal antibody (mAb: clone TDR31.1, mouse IgG1, a monomorphic HLA-class II β-chain epitope of HLA-DR+DQ+DP), fluorescence isothiocyanate (FITC)-conjugated HLA-class II mAb (mouse IgG1), and FITC-conjugated isotype-matched normal mouse IgG1 antibody (Ab) were purchased from Cosmo Bio. Co. (Tokyo).

Dendritic cells

The dendritic cells (Mylc-DCs) [6] used in the present study were obtained from MiCAN Technologies Inc. (Kyoto). The Mylc-DCs are powerful cell model of activated dendritic cells (DCs) and are established by the iPS cell technology (so-called regenerative medicine technology) at MiCAN Technologies Inc. It is possible to produce Mylc-DCs stably in large amounts, and the cells exhibit a very high sensitivity to various kinds of stimulants and substances. The cells were cultured in Mylc-DC-specific growth medium according to a routine protocol provided by MiCAN Technologies Inc. The morphology of the Mylc-DCs was observed and photos were taken under a phase-contrast microscope.

Analysis of the Mylc-DCs treated/not treated with HLA-class II mAb

The Mylc-DCs (2×10^5 cells/mL) were seeded in culture flasks (Sumitomo Co., Tokyo) containing growth medium and were treated/not treated with an optimal concentration of HLA-class II mAb (1.0μg/mL) or isotype-matched normal mouse IgG1 Ab (1.0μg/mL) in the presence/absence of an optimal concentration of the conventional protein kinase C (cPKC) inhibitor, Go6976 (3.0μM), and incubated for 24 hrs in a CO_2 atmosphere. After 24 hrs, the culture supernatants were collected and analyzed for the presence of interleukin-6 (IL-6) and IL-8 using enzyme-linked immunoassay (EIA) kits (Diaclone Co.). The measurements of IL-6 and IL-8 were repeated five times.

Flow cytometry

The cultured Mylc-DCs were washed in cold phosphate-buffered saline (PBS) containing 0.1% NaN_3 (hereinafter referred to as the washing buffer) and incubated in washing buffer containing 25% normal goat serum and 1 mg/mL of normal human IgG for 10 min on ice, to block the Fc receptor for IgG. The cells were then incubated with an optimal concentration of an FITC-conjugated HLA-class II mAb for 40 min at room temperature. A negative control (NC) was set up by incubation of the cells with FITC-conjugated isotype-matched normal mouse IgG1 Ab under the same conditions. Following a final wash with the washing buffer, the cellular debris was excluded, and the percentage of positively stained cells for HLA-class II mAb was determined based on their single-color flow-cytometric profile using a fluorescence activated cell sorter (FACScan) (Becton Dickinson Co.). The percentage of cells showing positive staining for the HLA-class II mAb was calculated. The experiment was carried out in triplicate per sample and repeated three times.

Statistical analysis

Data are expressed as the means ± standard deviation (SD). The statistical analysis was performed using an unpaired Student' s t-test. Differences at $P < 0.01$ were considered as being significant.

Results and Discussion

First, we observed the morphology of the Mylc-DCs under the phase-contrast microscope (Fig. 1A, a: 100x and b: 400x). Then, we investigated the expression pattern of the HLA-class II molecules on the Mylc-DCs using a flow-cytometric system. As shown in Fig. 1B, HLA-class II molecules were expressed on most (99.9%) of Mylc-DCs.

Next, we investigated the amounts of cytokines, such as IL-6 and IL-8, produced by the Mylc-DCs treated/not treated with an optimal concentration of HLA-class II mAb (1.0μg/mL) or isotype-matched normal mouse IgG1 Ab (1.0μg/mL) after 24 hrs treatment, using an EIA system. First, as shown in Figs. 2 and 3, IL-6 (72.56 ± 5.31 pg/mL) (Fig. 2) and IL-8 (1808.22 ± 67.74 pg/mL) (Fig. 3) were spontaneously produced by the Mylc-DCs and released into the culture supernatants at 24 hrs even in the absence of HLA-class II mAb or isotype-matched normal mouse IgG1 Ab treatment. On the other hand, the production of IL-6 (Fig. 2) and IL-8 (Fig. 3) and their levels in the culture supernatants (617.96 ± 14.59 pg/mL and 8828.91 ± 213.69 pg/mL, respectively) after 24 hrs treatment were found to be strongly enhanced in the cultures treated with HLA-class II mAb, but not in those treated with isotype-matched normal mouse IgG1 Ab. Although the data are not shown, the enhancement of IL-6 and IL-8 production by the HLA-class II mAb was dose-dependent. These data clearly indicate that the productions into the culture supernatants of both IL-6 and IL-8 in the Mylc-DCs were specifically and significantly ($P < 0.001$) enhanced by HLA-class II mAb treatment, as compared with those in the control Mylc-DCs (not treated with HLA-class II mAb or treated with isotype-matched normal mouse IgG1 Ab) (Figs. 2 and 3).

In addition, as shown in Figs. 2 and 3, the enhanced productions of IL-6 and IL-8 from the Mylc-DCs induced by HLA-class II mAb were significantly ($P < 0.001$) suppressed (IL-6, 318.95 ± 17.91 pg/mL; Fig.2, IL-8, 4248.43 ± 256.97 pg/mL; Fig. 3) in the presence of an optimal concentration of the cPKC inhibitor Go6976 (3.0μM).

DCs are derived from hematopoietic bone marrow progenitor cells and are characterized by high endocytic/phagocytic activity, but not strong T-cell activation potential [1-4]. DCs are constantly exposed to pathogens, such as viruses and bacteria, in their environment, which are recognized by pattern recognition receptors (PRRs) or toll-like receptors (TLRs). This results in the DCs becoming activated DCs expressing HLA-class II molecules (HLA-DR+DQ+DP) and migrating to the lymph nodes in humans. Here, they act as APCs to $CD4^+$ and $CD8^+$ T-cells, as well as to B-cells [7,8]. Foreign antigens such as pathogenic bacteria in the activated DCs expressing HLA-class II molecules are degraded by proteases in the cells to become peptide fragments, which are transported to and bind to the HLA-class II molecules. The resultant antigen-HLA-class II complex is transported to the cell surface, and the antigen is presented to the T-cell receptor (TCR) of $CD4^+$ or $CD8^+$ T-cells. In this immunological process, the HLA-class II molecules play important roles, however, the direct signal transduction pathway via the HLA-class II molecules expressed on the activated DCs is not yet understood.

In order to analyze the mechanism(s), we used the Mylc-DCs established by the iPS cell technology, or the so-called regenerative medicine technology, because DCs are only contained in minute amounts in the peripheral blood leukocytes, and it is difficult to obtain them in large quantities and in a stable manner. In addition, since it is derived from one donated blood, the quality is not stable, and there are issues with the reliability of the test evaluation. On the other hand, the Mylc-DCs exhibit very high sensitivity to various kinds of substances, and cells with the same genetic background can be produced stably in large amounts [6].

In this study, we first found that the Mylc-DCs that express HLA-class II molecules showed strongly enhanced IL-6 and IL-8 production following agonist

(HLA-class II mAb) stimulation, and the enhanced IL-6 and IL-8 productions from the Mylc-DCs mediated by the HLA-class II molecules were significantly suppressed in the presence of the PKC inhibitor Go6976. These findings indicate that the production of IL-6 and IL-8 in the Mylc-DCs is tightly controlled by some phosphorylated proteins through some intracellular signal transduction pathway mediated by PKC. We strongly presume that transcription of NF-κB by PKC might be involved. However, other PKs such as protein kinase A (PKA), protein tyrosine kinase (PTK), and also cyclic AMP (cAMP) and intracellular Ca^{2+} concentrations [9,10] may be involved in the production of IL-6 and IL-8 from the Mylc-DCs via the HLA-class II stimulation pathway. In order to verify this speculation, we are vigorously pursuing further experiments in various fields.

Further analyses are necessary for obtaining a better understanding of the intracellular signal transduction pathways in the Mylc-DCs at the molecular (mainly transcription of NF-κB) level.

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Disclosure

None of the authors has any conflict of interest to disclose.

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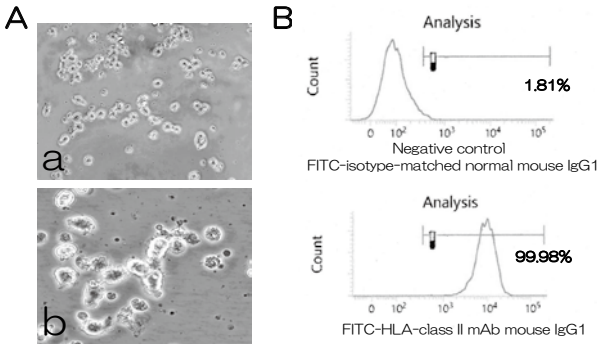

Figure 1. A: Morphology of the Mylc-DCs was observed by phase-contrast microscopy (a:100x and b: 400x). B: Typical expression pattern of HLA-class II molecules on the Mylc-DCs. The Mylc-DCs were incubated with an FITC-conjugated HLA-class II mAb for 40 min. The percentages of positively stained cells were analyzed using a single-color flow-cytometric system (See Materials and Methods for details). The percentages are shown in each panel. A negative control (NC) was set up by incubating the Mylc-DCs with an FITC-conjugated isotype-matched normal mouse IgG1 Ab. The analysis was repeated in triplicate for each sample, and the experiment was repeated three times.

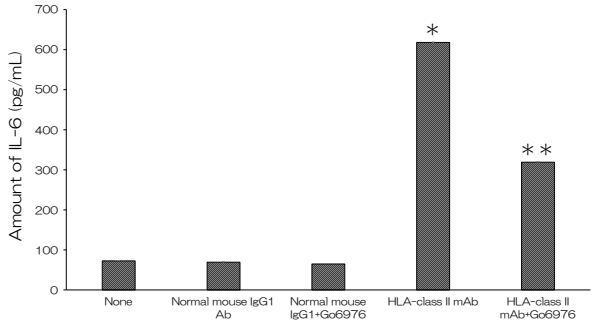

Figure 2. Detection of the amount of IL-6 produced from the Mylc-DCs treated/not treated with HLA-class II mAb (1.0μg/mL) or isotype-matched normal mouse IgG1 Ab (1.0μg/mL) in the presence/absence of an optimal concentration of a PKC inhibitor (Go6976). The amounts of IL-6 in the culture supernatants of the Mylc-DCs after 24 hrs treatment were determined using an EIA system. Data are expressed as means ± SD. The analysis was repeated in triplicate for each sample, and the experiment was repeated four times. *P < 0.001 (HLA-class II mAb vs. None). ** P < 0.001 (HLA-class II mAb vs. HLA-class II mAb+Go6976).

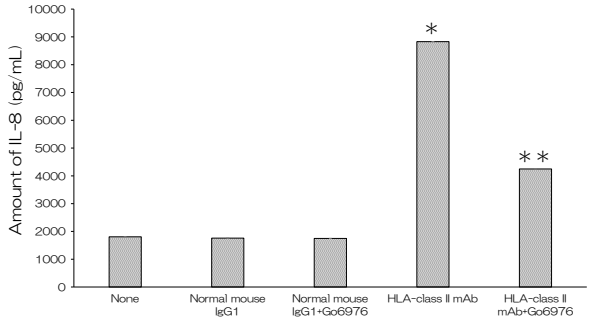

Figure 3. Detection of the amount of IL-8 produced from the Mylc-DCs treated/not treated with HLA-class II mAb (1.0μg/mL) or isotype-matched normal mouse IgG1 Ab (1.0μg/mL) in the presence/absence of an optimal concentration of a PKC inhibitor (Go6976). The amounts of IL-8 in the culture supernatants of the Mylc-DCs after 24 hrs treatment were determined using an EIA system Data are expressed as means ± SD. The analysis was repeated in triplicate for each sample, and the experiment was repeated four times. *P < 0.001 (HLA-class II mAb vs. None). ** P < 0.001 (HLA-class II mAb vs. HLA-class II mAb+Go6976).

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HLA-class II 抗体による iPS 細胞技術で確立した樹状細胞（Mylc-DCs）からのサイトカイン産生増強

池脇 信直　アブラハム サミュエル*

九州保健福祉大学生命医科学部生命医科学科・順正学園免疫学研究所　〒882-8508　宮崎県延岡市吉野町1714-1
*山梨大学医学部臨床研究推進センター 〒409-3898　山梨県中央市下河東1110

要旨

HLA-class II抗体によるiPS細胞技術で確立した樹状細胞(Mylc-DCs)からのサイトカイン産生能を解析した。Mylc-DCsはインターロイキン -6(IL-6）と IL-8を培養液中に自然産生していることがわかった。Mylc-DCsを至適濃度の HLA-class II抗体(1.0μg/mL）で処理したところ、IL-6と IL-8の産生は有意に増強した(P < 0.001)。次に、HLA-class II抗体で処理した Mylc-DCsに至適濃度の protein kinase C(PKC）活性阻害剤である Go6976（3.0μM）を添加したところ、IL-6と IL-8の産生増強は有意に抑制された(P < 0.001)。以上の結果から、HLA-class II抗体は Mylc-DCsの細胞内シグナル伝達経路を活性化すること、さらに、HLA-class II抗体で処理した Mylc-DCsからの IL-6と IL-8の産生増強は、PKCが密接に関与していることがわかった。

キーワード：サイトカイン、HLA- クラスⅡ抗体、Mylc-DCs、プロテインキナーゼ C