Evaluation of Adjuvant Activities Using Human Antigen Presenting Cells in Vitro

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ABSTRACT

Background: Immunomodulators such as lipopolysaccharides (LPS) and forskolin change the nature of dendritic cells (DCs) to induce Th1 and Th2 cells, respectively, thereby designated Th1 or Th2 adjuvants. Our previous study showed that such activities can be qualitatively evaluated by expression patterns of Notch ligand isoforms, using human monocyte-derived DCs and some leukemic cell lines, such as THP-1 or KG-1. However, quantitative evaluation of the adjuvant activities was not fully established.

Methods: PMA-treated human monocytic cell line THP-1 was used as a target. Cells were stimulated with various adjuvants, and the intracellular levels of cAMP were determined.

Results: Th2 adjuvant forskolin was qualitatively evaluated by an increased expression of Delta1 in PMA-treated THP-1 cells, as reported in our previous study. In PMA-treated THP-1 cells, intracellular cAMP levels increased after stimulation with forskolin, in a dose-dependent manner. On the other hand, LPS, one of the known Th1 adjuvants, suppressed the increased cAMP level in a dose-dependent manner.

Conclusions: Th2- and Th1-adjuvant activities can be quantitatively evaluated by using PMA-treated THP-1 cells and PMA-treated/forskolin-stimulated THP-1 cells, respectively.

KEY WORDS

adjuvant, cAMP, dendritic cells, Notch ligand, THP-1

INTRODUCTION

Dendritic cells (DCs) play a pivotal role in the differentiation of naïve CD4 T helper cells toward Th1 or Th2 cells. Environmental molecules, such as LPS, certain nucleic acids and fungus-derived glycoprotein molecules alter the DC function and induce Th1 differentiation.¹ Because such DCs induce Th1 responses, they are designated DC1, and DC1-inducing molecules are called Th1 adjuvants. DC2 and Th2 adjuvants have also been reported. DCs matured in the presence of forskolin or prostaglandin E2 (PGE2) induce the differentiation of naïve CD4 T cells toward Th2.² Moreover, induction of DC2 by PGE2 was associated with increased intracellular cAMP levels.³

Notch signaling pathways are highly conserved in organisms ranging from invertebrates to mammals and they are involved in cell fate choice during development.⁴ We have reported, by using human systems, a correlation of Notch ligand mRNA levels of monocyte-derived DCs (Mo-DCs) and leukemic APCs with Th1/Th2 adjuvant activities.⁵ Increased expression of Delta1 and Delta4 mRNA on DCs can predict Th2 and Th1 adjuvant activities, respectively. Indeed, in other studies using mouse models, Notch was directly regulated to Gata-3 expression during Th2 differentiation.⁶,⁷ In this study, by using human THP-1 cells, we quantitatively evaluated Th1/Th2 adjuvant activities.

METHODS

CELL

THP-1 cells were cultured in RPMI1640 media, supplemented with 10% fetal calf serum, 1% penicillin and
**RESULTS**

To determine whether a Th2 adjuvant forskolin exhibits Delta1-inducing activity, we observed Delta1 mRNA expression by using RT-PCR. As expected, forskolin but not LPS induced increased Delta1 mRNA expression on PMA-treated THP-1 cells (Fig. 1). Because LPS induced increased Delta4 expression in our previous study, such differential induction of Notch ligand isoforms can be used for a qualitative evaluation of Th1/Th2 adjuvants. However, quantitative evaluation of the activities requires real-time RT-PCR analysis, which is not suitable for a high-throughput assay. We therefore tried to use intracellular cAMP levels for the high-throughput assay, which have been reportedly to be increased by Th2 adjuvants.

As shown in Figure 2, mock-treated (PMA 0 ng/ml) THP-1 cells did not exhibit forskolin-induced cAMP elevation. In PMA-treated THP-1 cells, cAMP elevation was observed in a forskolin dose-dependent manner. It is noteworthy that at 50 ng/ml PMA, 5 μM forskolin most markedly induced cAMP elevation. Similar results were obtained from studies using PMA-derived KG-1 cells. However, cAMP elevation of KG-1 cells induced by forskolin was marginal. We therefore used PMA-treated THP-1 cells in the subsequent studies. The viability of THP-1 cells markedly decreased at 50–100 ng/ml PMA in the presence of 10 μM forskolin. Such forskolin-induced cAMP elevation at 50 ng/ml PMA can be used as target cells for Th1 adjuvants. Thus, as shown in Figure 3, PMA (50 ng/ml)-treated and forskolin (5 μM)-induced cAMP elevation was inhibited by LPS, in a dose-dependent manner, ranging from 0.01 to 10 μg/ml of LPS. The data was shown as mean value of duplicate determinations.

LPS and forskolin are known to be Th1 and Th2 adjuvants, respectively. Indeed, we have demonstrated that LPS and forskolin antagonized each other, by using MLR systems (data not shown). In the present study, forskolin dose-dependently induced intracellular cAMP levels in PMA-treated THP-cells. Furthermore, Delta1 mRNA expression was increased in a forskolin-dose-dependent manner (data not shown), which is indicative of the availability for the quantitative and qualitative evaluation of Th2 adjuvant activities. The forskolin-induced cAMP elevation was successfully antagonized by LPS, again in a dose-dependent manner, which is indicative of the availability for the quantitative evaluation of the Th1 adjuvant activity.
In Vitro Evaluation of Adjuvants

DISCUSSION

The detection of Th1/Th2 adjuvant activities has long been dependent on an assay for the cytokine profiles induced on T cells by co-culturing with APCs stimulated with adjuvants. This assay requires a large amount of labor and more than 2 weeks due to the preparation of cells from healthy donors and a large number of culture processes. In addition, the heterogeneities of cell sources and donor-to-donor variances may also lead to difficulties in obtaining stable and reproducible results. The present study demonstrated for the first time that measurement of intracellular cAMP in PMA-derived THP-1 cells have the potential to scrutinize and evaluate Th1/Th2 adjuvant candidates out of a large number of environmental substances and natural products, without possible instability arising from cell sources and polymorphisms. It is also of note that this assay system can be used for the evaluation of live bacteria, because 3-hour incubation with live probiotic bacteria did not decrease the viability of THP-1 cells and successfully induced Delta1 and Delta4.

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REFERENCES

2. Kalinski P, Schuitemaker JH, Hilkens CM, Kapsenberg ML. Prostaglandin E2 induces the final maturation of IL-12-deficient CD1a+CD83+ dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. *J Immunol* 1998;161:2804-9.