Food Chemistry 138 (2013) 757–761

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Apple polyphenols suppress antigen presentation of ovalbumin by THP-1-derived dendritic cells

Shigeru Katayama [a], Takuya Kukita [a], Eri Ishikawa [a], Syohei Nakashima [b], Susumu Masuda [b], Tomomasa Kanda [b], Hiroshi Akiyama [c], Reiko Teshima [c], Soichiro Nakamura [a,*]

[a] Department of Bioscience and Biotechnology, Shinshu University, 8304 Minamiminowamura, Ina, Nagano 399-4598, Japan
[b] Research Laboratories for Fundamental Technology of Food, Asahi Group Holdings, Ltd., 1-1-21, Midori, Moriya, Ibaraki 302-0106, Japan
[c] National Institute of Health Sciences, 1-8-1, Kamiyoga, Setagaya, Tokyo 158-8501, Japan

ARTICLE INFO

Article history:
Received 15 June 2012
Received in revised form 25 August 2012
Accepted 1 October 2012
Available online 17 November 2012

Keywords:
Antigen presentation
Dendritic cells
THP-1
Polyphenol
Procyanidin

ABSTRACT

Apple polyphenol extract (AP) and procyanidin contained in AP were investigated for their immunomodulatory effects using THP-1-derived human dendritic cells (TDDCs). The expression levels of HLA-DR (MHC class II) and CD86 (costimulatory molecule) were measured as an indicator of antigen presentation in TDDCs. A significant decrease in HLA-DR expression was observed in the AP and fractionated procyanidin-treated cells in the presence of ovalbumin (OVA), but no effect on CD86 expression was observed. The uptake of OVA was not inhibited by AP treatment, and the gene expression of membrane-associated RING-CH ubiquitin E3 ligase, MARCH1, was up-regulated by AP treatment. It can therefore be presumed that AP suppresses HLA-DR expression *via* the ubiquitin–proteasome pathway. Furthermore, the up-regulation of IL-12 and TNF-α was found in the procyanidin trimers-treated cells in the presence of OVA. These results suggest that apple polyphenols would be an effective factor for the development of immunomodulatory agents with suppressive effects of antigen presentation.

1. Introduction

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) located in tissues such as skin and mucous membranes and play a critical role in activating T cell-dependent immune responses (Banchereau & Steinman, 1998). DCs are also likely to be essential for the initiation of allergic immune responses. DCs uptake exogenous antigens, process them into immunogenic peptides, and express these peptides for presentation onto major histocompatibility complex (MHC) class II molecules to initiate immune responses. It has also been postulated that DCs are important for the induction of immunologic tolerance (Shortman & Heath, 2001).

Recently, it has been pointed out that dietary factors might contribute to the prevention and treatment of allergic diseases. Typically, the anti-allergic effects of probiotic bacteria, prebiotics, and dietary polyphenols have been demonstrated (Johannsen & Prescott, 2009; Rozy, Jagus, & Chorostowska-Wynimko, 2012; Singh, Holvoet, & Mercenier, 2011). Akiyama et al. (2005) reported that apple polyphenol extract (AP) inhibits the development of food allergies in murine models. AP is mixture of oligomers consisting of chains of flavan-3-ol subunits mainly joined through C4–C8 (or C6) bonds (Ohnishi-Kameyama, Yanagida, Kanda, & Nagata, 1997). Furthermore, it has been shown that AP had an inhibitory effect on histamine release from rat basophilic leukaemia cells by antigen stimulation (Kanda et al., 1998; Nakano et al., 2008) and that AP intake improved the symptoms of atopic dermatitis in the patients (Kojima et al., 2000). It is presumed that AP has a direct suppressive effect on antigen presentation by APCs such as DCs by inhibiting the cell surface expression of MHC class II and co-stimulatory molecules, resulting in inefficient antigen presentation, and exerts an anti-allergic effect. However, the direct effect of AP on DCs' function remains unknown.

The current protocol to study the biological function and role of DCs is mainly through differentiation of human donor-derived peripheral blood monocytes with cytokines such as GM-CSF and IL-4 (Casati et al., 2005). Several cell lines including monocytic THP-1 and KG-1 cell lines have been also used as cellular models (Berges et al., 2005). Although a number of studies have been reported for the assessment of the sensitizing potential of chemical allergens using *in vitro*-generated DCs (dos Santos et al., 2009), there are a few studies for the evaluation of allergenicity of food proteins and the screening assay of immunomodulatory agents (Hilmenyuk et al., 2010; Shreffler et al., 2006). The newly developed DCs should be useful tools for quick and easy screening of anti-allergic agents with suppressive effect on antigen presentation. Based on the above theoretical background experimental

* Corresponding author. Tel./fax: +81 265 77 1609.
E-mail address: snakamu@shinshu-u.ac.jp (S. Nakamura).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.10.076

studies of DCs, we successfully obtained DCs from THP-1 cell lines by culturing with PMA and IL-4, and then investigated the effects of AP on antigen presentation of ovalbumin (OVA) and cytokine secretion in THP-1-derived DCs (TDDCs) in this study.

2. Materials and methods

2.1. Materials

AP (procyanidin oligomers, 65.7%; monomeric flavan-3-ols, 12.5%; chalcones, 6.5%; phenolcarboxylic acids, 10.8%) and each procyanidin oligomer fraction (monomers to octamers) (Shoji, Masumoto, Moriichi, Kanda, & Ohtake, 2006) were kindly provided by Asahi Group Holdings (Ibaraki, Japan). Recombinant human interleukin 4 (IL-4) was purchased from PeproTech (Rocky Hill, NJ). Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma (St. Louis, MO). Other reagents used for flow cytometric analysis were purchased from BD Biosciences (San Jose, CA). All other reagents were of biochemical grade.

2.2. Cell culture and differentiation

Human acute monocytic leukaemia cell line, THP-1, was obtained from JCRB cell bank (Osaka, Japan) and maintained in RPMI 1640 medium supplemented with 10% foetal bovine serum (FBS), 50 U/ml penicillin, and 50 μg/ml streptomycin (Wako, Osaka, Japan) at 37 °C in a humidified atmosphere with 5% CO_2 in air. After culturing from the cell stock, THP-1 cells were used under 10 passages to minimize variability. To induce differentiation into DCs, THP-1 was seeded at 5×10^5 cells/well in a six-well plate and incubated in 2 ml/well in RPMI 1640 medium supplemented with PMA (final concentration, 20 ng/ml) and IL-4 (10 and 20 ng/ml). On day 4, the resulting immature DCs were washed with PBS, stimulated with different concentrations of OVA (0.1, 0.5, and 1 mg/ml) in RPMI 1640 medium supplemented with 10% FBS, 50 U/ml penicillin, and 50 μg/ml streptomycin, and further incubated for 72 h in the absence or presence of AP (10, 50, and 100 μg/ml) and fractionated procyanidins (50 μg/ml).

2.3. Flow cytometry analysis

Expression of cell surface markers was analysed by flow cytometry. Cell staining was performed using the following fluorescence-labelled anti-human monoclonal antibodies (mAbs): FITC-conjugated CD86 (clone BU63) from AbD Serotec (Oxford, UK), Alexa Fluor 647-conjugated HLA-DR (clone L243), CD209 (clone 9E9A8), PerCP/Cy5.5-conjugated CD11c (clone 3.9), and mouse IgG isotype controls from BioLegend (San Diego, CA). Cells were harvested with 1 mM EDTA in PBS, washed with PBS, then incubated with mAbs and appropriated isotypic controls using the manufacturer's recommended dilutions at 4 °C for 30 min after blocking the Fc receptor with FcR blocking reagent (Miltenyi Biotec, Auburn, CA) at 4 °C for 15 min. The stained cells were washed, resuspended with PBS containing 1% BSA, and analysed using FACSCalibur and CellQuest software (BD Bioscience). A total of 10,000 cells were analysed. Expression levels were expressed as delta mean fluorescence intensity (MFI), which was calculated as: MFI of the cells stained with fluorochrome-conjugated antibody − MFI of the background staining cells.

2.4. Analysis of OVA uptake

The immature TDDCs were washed and resuspended in culture medium containing 1 mg/ml FITC-labelled OVA (Sigma) and incubated for 30 min at 37 °C. The cells were washed three times in cold PBS and then analysed with a FACSCalibur flow cytometer. Uptake of OVA was measured as FITC mean fluorescence intensity (MFI) compared to the control cells. The uptake of FITC-labelled OVA at 4 °C was measured as a negative control.

2.5. Real-time PCR

Total RNA was isolated from the control and AP-treated cells using RNAiso plus Kit (TaKaRa, Japan), and cDNA was generated using the High Capacity RNA-to-cDNA Kit (Applied Biosystems, Carlsbad, CA). cDNA was used as a template for real-time PCR in triplicates with Fast SYBR Green Master Mix and gene-specific primers. PCR and analysis were performed on a StepOne Real-time PCR system (Applied Biosystems). Primer sequences were as follows: β-actin (forward) 5′-CACTATTGGCAACGAGCGGTTC-3′, β-actin (reverse) 5′-ACTTGCGGTGCACGATGGAG-3′, membrane-associated RING-CH (MARCH) 1 (forward) 5′-TCCCAGGAGCCAGTCAAGGTT-3′, MARCH1 (reverse) 5′-CAAAGCGCAGTGTCCCAGTG-3′, MARCH2 (forward) 5′-CTCAGCCTCCCAAGTAGCTG-3′, MARCH2 (reverse) 5′-CTT GAGGCCAGGACTTTGAG-3′, MARCH8 (forward) 5′-ACAGGAAGC CTCCACTTCG-3′, MARCH8 (reverse) 5′-GACGTGGAATGTCACTGAG-3′. Cycling was initiated at 95 °C for 1 min, followed by 40 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min. The relative expression of each gene was calculated using the comparative threshold cycle method normalized to β-actin.

2.6. Quantification of cytokine production by ELISA

Culture supernatants were collected after 72 h sample treatment and stored at −20 °C until use. The production of cytokines (IL-12 p70, IL-1β, TNF-α, and IL-10) was measured by ELISA kit (Bio-Rad Laboratories, Hercules, CA) using 96-well plates according to the manufacturer's instructions.

2.7. Statistical analysis

The results were expressed as the means ± SD. Statistical evaluation was carried out using an unpaired Student's *t*-test with Statcel software ver. 2.0 (OMS-Publishing, Saitama, Japan).

3. Results

3.1. Generation of immature DCs from THP-1 cells

Human monocytic cell lines, THP-1, were differentiated into immature DCs by culturing with PMA and IL-4 for 4 days. The obtained adherent cells had a typical dendritic morphology (data not shown), and significant expression of CD11c and CD209 (DC-SIGN), surface markers indicative of DCs, was observed in PMA/IL-4 treated cells with the dose dependence of IL-4 (Fig. 1). It was named THP-1-derived DCs (TDDCs) obtained by culturing for 4 days with 20 ng/ml PMA and 20 ng/ml IL-4 and used for further experiments.

3.2. Suppressive effect of AP on antigen presentation by TDDCs

We next investigated whether AP would affect the antigen presentation on TDDCs stimulated with OVA. The expression of MHCII subunit (HLA-DR) and co-stimulatory molecule (CD86) was measured as an indicator of antigen presentation. When TDDCs were stimulated with 1 mg/ml OVA, 1.4- and 1.6-fold increases in the expression of HLA-DR and CD86, respectively, were observed compared with the control cells (Fig. 2). The effect of AP treatment on the expression levels of HLA-DR and CD86 in 1 mg/ml OVA-stimulated TDDCs was then investigated. As shown in Fig. 3, the expression level of HLA-DR was significantly decreased in the TDDCs