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Induction of Rice Allergen-Specific IgE Synthesis by KU812 Cells

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In vitro IgE class switching could be induced through co-culture of CD40L-expressing KU812 cells and CD40-expressing B cells in the presence of IL-4 or IL-13. It has been generated several B cell lines, which produce rice allergen (RA)-specific IgM antibody by *in vitro* immunization (IVI) using peripheral blood lymphocyte (PBL). In this study, induction of RA-specific IgE antibody by KU812 cells was attempted. Before co-culture, we determined the CD40 expression in RA-specific B cell lines, RA9G11 and the CD40 ligand (CD40L) expression in activated KU812 cells by treatments with phorbol myristate acetate (PMA) and ionomycin for 6 hrs. Flow cytometric analysis shown that RA9G11 and activated KU812 cells expressed high level of CD40 and CD40L, respectively. RA9G11 cells were cultured with activated KU812 cells for 12 days in the presence of IL-4 for IgE class switching. Mature Cε mRNA level and RA-specific IgE spot forming cells (SFC) were observed in all culture condition, and especially, high level of RA-specific IgE synthesis was determined the same ratio of RA9G11 and activated KU812 cells in the presence of 50U IL-4. Therefore, induction of RA-specific IgE synthesis by activated KU812 cells can be contributed in the application for allergic therapy and prevention.

Key words : IgE, rice allergen (RA), KU812, RA9G11

Introduction

Immunoglobulin E (IgE) is plays an important role in allergic diseases. IgE production in B cells requires a physical interaction with activated Th2-type $CD4^+$ T cells, which produce a number of IL-4 or IL-13, and expressed CD40L [3,4,6,14,15]. Ligation of CD40 on B cells with CD40L results in enhancement of germ-line Cε transcription induced by IL-4 or IL-13 and subsequent isotype switching to IgE antibody, which leads to mature Cε gene transcription and IgE synthesis. It is reported that mast cells (HMC-1) and basophils (KU812) express the CD40L in response to immunologic or pharmacologic stimulation, and can be replaced by $CD4^+$ T cells, which is shown to be responsible for the IgE synthesis [2,10,13]. Regardless of the cell type, CD40L expressing cells can induce IgE synthesis in B cells in the presence of IL-4 or IL-13. Yanagihara et al reported that human IgE synthesis in B cells was induced by basophilic cell line KU812, which stimulated with PMA and ionomycin [19]. KU812 cells, which have been described as a human leukemia derived cell lines, are generally accepted to be an immature basophilic cell line expressing a low level of high affinity IgE receptor [2,4,8,10,13,19]. A number of studies reported that induction of differentiation has been used the KU812 cells as a model of human basophilic cell line [5,17,18].

The worldwide prevalence of allergic disease such as food allergy has dramatically increased with changes in life style and environmental pollution and increasing stress, particularly in developed countries [1,11]. The clinically important allergen sources in food are known to be eggs, milk, legumes, seafood, cereal, and meat. Rice is consumed and produced in large quantities around the world, and has been thought to be a source of atopic dermatitis in some adult patients, although the frequency of such rice allergy is lower that of egg, milk or soybean allergies [7,9,12]. We generated RA-specific IgM producing B cell line, RA9G11 by IVI using human PBL [9,16]. Therefore, in this study, we attempted class switching of RA-specific IgM antibody to IgE antibody.

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Materials and Methods

Reagents

RPMI-1640 medium was purchased from Nissui Pharmaceutical Co. (Tokyo, Japan). Fetal bovine serum (FBS) and antibiotics and antimycotic were purchased from Gibco BRL (Gaithersburg, MD, USA). Phorbol-12-myristate-

13-acetate (PMA) and ionomycin were purchased from Calbiochem (CA, USA). FITC (flourescein isothiocyanate)-conjugated anti-human CD154 (CD40L) antibody purchased from AnCell (Bayport, MN, USA). Horseradish Peroxidase (HRP) Conjugate anti-human IgE and FITC-conjugated anti-human CD40 antibodies were purchased from Biosource (Burlingame, CA, USA) TRI_{ZOL} reagent, oligo $(dT)_{20}$ primer and Superscript II reverse transcriptase were purchased from Invitrogen (Carlsbad, CA, USA). Taq DNA polymerase was purchased from Roche (Mannheim, Germany). All other reagents, such as hydroxyethyl piperazinylethanesulfonic acid (HEPES), L-glutamine, and diethylpyrocarbonate (DEPC) were purchased from Sigma Chemicals (St. Louis, MO, USA).

Cell culture

KU812 cells were obtained from Japanese Collection of Research Bioresources (JBRC) and cultured in RPMI-1640 medium. RA-specific IgM producing RA9G11 cells were cultured in ERDF medium. The media were supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 10 mM HEPES buffer, 100 μg/ml of streptomycin and 100 U/ml of penicillin, and cultured at 37°C in a humidified atmosphere with 5% CO_2 and passaged every 3-4 days.

Induction of CD40L expression

KU812 cells (1×10^6 cells) were stimulated with 10 ng/ml of PMA and 1 μM of ionomycin for 6 hrs [19]. The stimulated cells were fixed with 4% p-formaldehyde for 30 min on ice.

Flow cytometric analysis

To analyze the CD40 expression in RA-specific IgM producing cells, RA9G11 cells were stained with 10 μg/ml of FITC-conjugated anti-human CD40 antibody. And the activated KU812 cells were stained with 20 μg/ml of FITC-conjugated anti-human CD40L antibody. Mouse IgG was used as negative control. The stained cells were analyzed by flow cytometry.

Cell culture for IgE class switching

RA9G11 cells were co-cultured with KU812 cells, which were fixed by 4% p-formaldehyde after stimulation with PMA/Ionomycin. The cells were cultured in ERDF medium supplemented with 10% FBS and 10% supernatant of RA9G11 in the presence of recombinant IL-4 for 12 days.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated using TRI_{ZOL} reagent according to the manufacturer's instruction. For cDNA synthesis, total RNA was reversely transcribed using a oligo $(dT)_{18}$ primer and reverse transcriptase. The resultant cDNA samples were subjected to PCR amplification in the presence of specific sense and antisense primers. Human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as an internal control. The primer sequences were as follow: for the Cε, sense 5′-TCGAETTETGGGGCCAAGGG-3′ and antisense 5′-GCCAGGTCCACCACCAGACAGGTGC-3′; for the G3PDH, sense 5′-CTCAGACACCATGGGGAAG G-3′ and antisense 5′-TGGTGCAGGAGGCATTGCTG-3′. The PCR was performed as following condition; denaturation, at 94°C for 1 min; annealing, at 60°C for 1 min and extension, at 65°C for 2 min and subjected to 25 and 35 cycles for G3PDH and Cε gene, respectively. The amplified DNA products were visualized by 1% agarose gel electrophoresis.

Enzyme-linked immunospot (ELISPOT) assay

Multiscreen HA plate was coated with RA, and blocked with 5% FBS/PBS. The cells were added to individual wells (1×10^3 to 1×10^5 cells/well) and incubated for 2 days. The plate treated with 2 μg/ml of HRP-conjugated anti-human IgE antibody. And then spots representing single IgE secreting cells were developed with the HRP substrate. The reaction was stopped by addition of distilled water, and the plate was washed with distilled water and dried by air. The spot was observed by microscopy.

Results and Discussion

CD40 and CD40L expression

It is known that the CD40-CD40L interaction plays a role in immunoglobulin class switching. KU812 cells are known to be a human basophilic cell line, which expresses CD40L corresponding to pharmacologic stimulation in the presence of IL-4 [8,19]. We generated RA-specific B cell lines, RA9G11, which produce IgM antibody, by IVI using PBL. In this study, class switching of RA-specific B cell line, RA9G11 to IgE producing cells by CD40L-expressed KU812 cells as class switching inducer was attempted (Fig. 1). Before the co-culture of RA9G11 and activated KU812 cells, we determined the CD40 and CD40L expression using flow cytomertic analysis. RA-specific B cell line, RA9G11

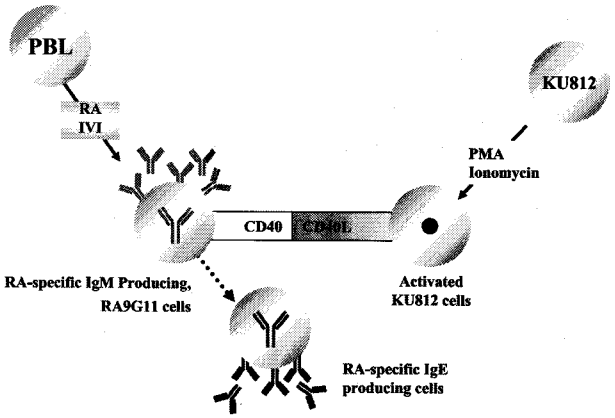

Fig. 1. Schematic representation for class switching of RA-specific IgM into IgE antibody

cells expressed 65.5% of CD40. KU812 cells, which is stimulated PMA/ionomycin for 6 hrs, could transiently up-regulate to 44.0% of the CD40L expression level (Fig. 2C). Therefore, activated KU812 cells could contribute to induce the antibody class switching.

Induction of IgE synthesis in B cells by KU812 cells

The activated KU812 cells could induce synthesis of IgE in B cells in the presence of exogenous recombinant IL-4. KU812 cells were stimulated with PMA/ionomycin for 6 hours and then fixed with 4% p-formaldehyde. RA-specific B cells, RA9G11 were cultured with stimulated and fixed KU812 cells for 12 days in the presence of various concentration of recombinant IL-4. Mature Cε mRNA level was determined by RT-PCR (Fig. 3). The Cε mRNA expression was up-regulated by co-culture with RA9G11 cells and

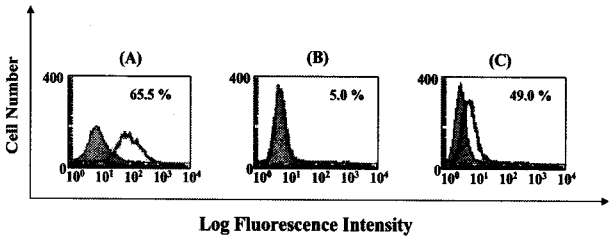

Fig. 2. CD40 and CD40L expression. RA-specific IgM producing RA9G11 cells were stained with FITC-conjugated anti-human CD40 antibody. KU812 cells were stimulated with PMA and ionomycin for 6 hrs and then stained with FITC-conjugated anti-human CD40L antibody. Stained cells were then analyzed by flow cytometry. Mouse IgG antibody was used as isotype-matched negative control. A, RA9G11; B, KU812; C, activated KU812 with PMA and ionomycin.

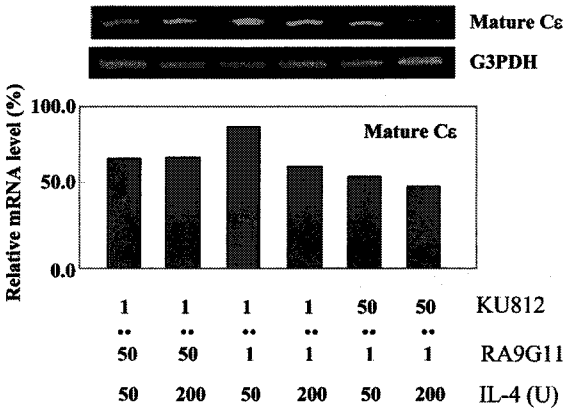

Fig. 3. Mature Cε mRNA level in class switched cells. RA9G11 cells were cultured with stimulated and fixed KU812 cells for 12 days in the presence of various concentration of recombinant IL-4, and cellular total RNA was isolated from class switched cells, and mature IgE constant region and G3PDH mRNA levels were analyzed by RT-PCR.

activated KU812 cells in the presence of IL-4 (Fig. 3). Furthermore, antigen-specificity against IgE antibody produced by co-culture was evaluated by ELISPOT assay. As shown in Fig. 4, RA-specific IgE SFC observed in all culture condition. Especially, increment of RA-specific SFC was determined in equal cell density of KU812 against RA9G11 in the presence of IL-4 (50 U/ml). These results known that RA-specific IgE synthesis was induced by co-culture of the same ratio cells in the presence of 50U IL-4, and CD40L on activated KU812 cells is functionally

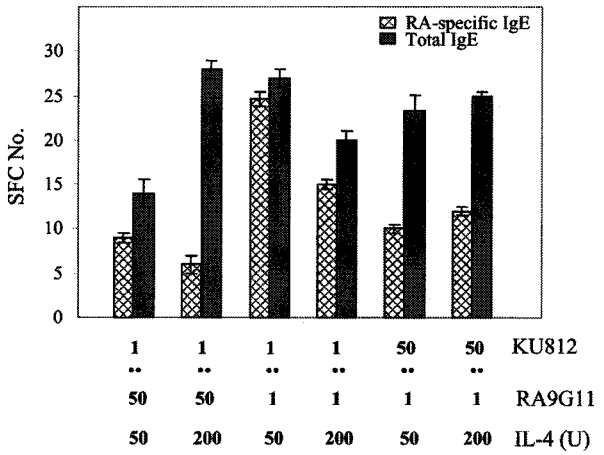

Fig. 4. Determination of RA-specific IgE antibody producing cells in class switched cells. RA9G11 cells were cultured with stimulated and fixed KU812 cells for 12 days in the presence of various concentration of recombinant IL-4, and SFC in class switched cells were determined by ELISPOT assay.

active, and could induce class switching to antigen-specific IgE antibody. KU812 cells are known to be a human basophilic cell line, and CD40L expression is up-regulated in KU812 cells with treatment of pharmacologic stimulators [19]. Therefore, we thought that KU812 cells could contribute to antibody class switching. We determined that synthesis of RA-specific IgE antibody was induced by co-culture of high level of CD40 expressing RA-specific B cell lines, RA9G11 and CD40L expressing KU812 cells through analysis of Cε mRNA level and antigen-specificity. These results indicate that activated KU812 cells were able to induce mature Cε transcription and IgE synthesis in B cells in the presence of recombinant IL-4. The information about the RA-specific IgE antibody, which might trigger the IgE-mediated allergic reaction, would useful to establish a specific diagnosis and immunotherapy against rice allergy. Moreover, induction of cellular-based IgE producing cells could contribute to research about therapy and prevention of allergic diseases.

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초록 : KU812세포에 의한 쌀 알레르겐 특이적 IgE항체 합성의 유도

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CD40L를 발현하는 KU812세포와 CD40를 발현하는 항원 특이적 B 세포의 배양을 통해 IgE 클라스 스위치가 유도된다. 본 연구자는 체외면역법에 의해 건강인의 말초혈액을 이용하여 쌀 알레르겐 특이적 IgM 항체를 생성하는 B 세포주를 수립하였다. 본 연구에서는 KU812세포에 의한 쌀 알레르겐 특이적 IgE 항체의 유도를 시도하였다. 배양 전, 쌀 알레르겐 특이적 B 세포주, RA9G11과 PMA와 ionomycin을 6시간 처리하여 활성화된 KU812세포에서 CD40와 CD40L 발현량을 flow cytomerty를 통화여 각각 확인하였다. RA9G11세포와 활성화된 KU812세포는 높은 수준의 CD40와 CD40L를 각각 발현하였다. 쌀 알레르겐 특이적 IgE항체 합성 유도를 위해, 여러 가지 농도의 IL-4 존재하에, RA9G11세포와 활성화된 KU812세포를 12일 동안 배양하였다. IgE 정상영역의 mRNA 발현량과, 쌀 알레르겐 특이적 IgE 항체 생성 세포가 모든 조건에서 확인되었으며, 특히 50U의 L-4 농도 하에서 같은 비율의 RA9G11과 활성화된 KU812세포를 배양했을 때, IgE 합성이 가장 높았다. 따라서 활성화된 KU812세포에 의한 쌀 알레르겐 특이적 IgE 합성은 알레르기 질환의 치료 및 예방에 관한 연구에 기여할 것으로 사료된다.