

Short Communication

Production of IgE antibody to Japanese cedar pollen allergen Cry j1 by short term culture of human peripheral blood lymphocytes

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Abstract. Peripheral blood lymphocytes (PBL) from a patient allergic to Japanese cedar pollens, were stimulated with IL-4, IL-13, CD40-Ligand and/or hydrocortisone in the presence of Epstein-Barr virus in 96-well round bottomed culture plates, and the secretion of IgE-class antibody against a Japanese cedar pollen allergen Cry j1 in the supernatants were examined. PBL cultured with IL-4, and IL-4 + CD40-Ligand showed the highest IgE secretion and the cultures were maintained for 30 days. However, we failed to expand the culture with high IgE secretion. It was suggested that patient's PBL stimulated with IL-4 were useful for short term IgE production to Cry j1.

Keywords: IgE, cedar pollen allergen, Cry j1, IL-4, peripheral blood lymphocytes

1. Introduction

A one-tenth of populations were considered to suffer from allergy caused by Japanese cedar pollen (pollinosis) in Japan. There are several allergens in the cedar pollens and the most major allergen is Cry j1 located in so called Ubish body of the pollen particles [1–5]. In the previous study [6], we established human-mouse hybridomas secreting IgM class anti-Cry j1 human monoclonal antibodies from patients' peripheral blood lymphocytes (PBL) by infection with Epstein-Barr virus (EBV) followed by cell fusion with mouse myeloma cells.

While we showed epitopes on the primary structure of Cry j1 recognized by IgM class monoclonal antibodies,

IgE class human monoclonal antibodies seems to be more useful not only for analysis of epitopes but also allergy model experiment using cultured cells and animals. Additionally, IgE class antibodies are expected to have stronger affinity to the allergens by the mutation of Ig-variable region at the class switch from IgM to IgE.

In this study we attempted to achieve short term production of IgE antibody to Cry j1 by PBL stimulated with IL-4, IL-13, CD40-Ligand, or hydrocortisone in the presence of EBV.

2. Materials and methods

2.1. Preparation of human peripheral lymphocytes

Fourteen (14) volunteers were subjected to the IgE antibody titer analysis with Immuno-Fast-Check J1 (Mitsubishi Kagaku Iatron, Inc., Japan) for airborne al-

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lergens including Japanese cedar pollen, cat skin, and house dust mite. One patient (#14) with the highest titer for the cedar pollen allergen was chosen for further experiment.

Peripheral blood lymphocytes (PBL) were separated by discontinuous gradient centrifugation over Ficoll-Paque PLUS (GE Healthcare Life sciences, Tokyo) from heparinized peripheral blood. PBL were washed three times with enriched-RDF medium (ERDF, Kyokuto-Seiyaku, Japan) by centrifugation for three times.

This study was carried out according to the ethical code for human experiment of National Food Research Institute with informed consent before the first analysis of blood antibodies.

2.2. Stimulation of lymphocytes

PBL ($0.5 - 10 \times 10^4/\text{ml}$) were suspended in ERDF medium supplemented with 10% fetal calf serum (FCS), 10% EBV conditioned medium (supernatant of marmoset B95-8 cells), and Cyclosporin A ($2 \mu\text{g}/\text{mL}$), and aliquot (0.1 ml) were cultured in round bottomed 96 well microculture plates with cytokines, CD40-Ligand (CD40L), and hydrocortisone (HC). The concentration of recombinant human IL-4 (Strathmann Biotec AG, Germany), recombinant human IL-13 (Strathmann Biotec AG, Germany), recombinant human soluble CD40L (Biosource International Inc., USA), and HC were 100–400 unit/mL, 100 unit/mL, 10 ng/mL, and $1 \mu\text{mol}/\text{L}$ respectively. PBL were cultured for two weeks and the supernatants were analyzed for IgE antibodies against Cry j1 by ELISA.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Sixty (60) μl of Cry j1 solution ($2 \mu\text{g}/\text{ml}$, Seikagaku-Kogyo, Japan) diluted with $0.05 \text{ mol}/\text{L}$ NaHCO_3 were pipetted into 96-well micro ELISA plate (Sumitomo Bakelite, Japan) and the plates were incubated at 4°C for 16 hr. After discarding allergen solution, the wells were blocked with Block Ace (milk based blocking agent, Dainippon Sumitomo Pharma, Japan) for 1 hr. Then the wells were washed with phosphate buffered saline containing 0.05% Tween 20 (PBS-Tween) for three times, and the supernatants from PBL culture were pipetted into the wells and incubated at room temperature for 1 hr. IgE antibodies bind to Cry j1 on the wells were detected with horse radish peroxidase conjugated anti-human IgE antibodies ($1/2,000$ dilution, Biosource International Inc., USA) with 2,2' azino-di-

Table 1
Secretion of IgE antibody to Cry j1 by human peripheral blood lymphocytes stimulated with cytokines and EBV

Cytokines	Number of wells with IgE IgE concentration (A405nm)		
	< 0.05	< 0.1	0.1 <
None	74	24	0
IL-4	16	80	0
IL-4, HC	64	32	0
IL-4, CD40L	14	72	0
IL-4, IL-13, CD40L	94	2	0

PBL (1×10^4 cells in $100 \mu\text{L}$ -10%FCS-ERDF medium/well) from donor #14 were cultured in round bottomed 96 well plates with IL-4, IL-13, CD40L, or HC in the presence of EBV for 14 days.

(3-ethyl benzthiazolin sulfonic acid) (ABTS) as an enzyme substrate. The color developed was measured at 405 nm by V-Max micro plate reader (Molecular Devices, USA)

3. Results and discussion

In this study, we cultured PBL from a patient allergic to Japanese cedar pollen with IL-4 [7–9], IL-13 [10, 11], CD40L [12], or HC [11,12] to induce secretion of IgE class antibody to a cedar pollen allergen Cry j1. Table 1 shows the occurrence of anti-Cry j1 IgE antibodies in the supernatants from patient's PBL ($1 \times 10^4/\text{well}$). While the highest IgE secretion in one well was observed at IL-4 + CD40L culture, higher occurrence at an absorbance ratio from 0.05 to 0.1 was obtained at the culture with only IL-4. IL-4 + CD40L also gave relatively high occurrence of IgE production. To clarify the effect of IL-4, the low density culture of PBL with only IL-4 were performed. As shown in Table 2, 100 unit/ml of IL-4 gave the highest occurrence of IgE secreting wells against Cry j1 at the cell density of $1 \times 10^3/\text{well}$.

IL-4 is known to induce class-switch of Ig secretion from IgM to IgE in peripheral lymphocytes of mice and humans [7,8]. In this paper, we showed that stimulation of PBL only with IL-4 gave the highest occurrence of anti-Cry j1 IgE production. IL-13, CD40L, and HC are reported to contribute class switch of Ig heavy chain gene to induce IgE secretion, whereas those conditions did not increase IgE secretion in the experiment in Table 1. During the culture, the EBV-transformed cells with cell aggregations were observed under the microscopic observation, and we could maintain the EBV-transformed cells for 30 days by feeding medium containing IL-4. However, finally we lost all the

Table 2
Secretion of IgE antibody to Cry j1 by human peripheral blood lymphocytes stimulated with IL-4 and EBV

Cells/well	IL-4 (U/mL)	Number of wells with IgE IgE concentration (A405nm)		
		< 0.05	< 0.1	0.1 <
500	100	82	110	0
	200	80	112	0
	400	82	110	0
1000	100	62	129	1
	200	90	102	0

PBL (500 or 1000 cells in 100 μ L-10%FCS-ERDF medium/well) from donor #14 were cultured in round bottomed 96 well plates (2 plates each) with IL-4 and EBV for 14 days.

IgE production during the culture. IgE production of IL-4 induced and EBV-transformed PBL seemed to be unstable. Though, we concluded that, stimulation of patient's PBL with IL-4 and EBV was useful for the short term production of IgE to Cry j1.

For the analysis of pollen allergens, specific serum from allergic patients seems to be very useful to identify allergic proteins and antibody binding sequences (epitopes). However, the titers of the sera vary, it seems to be difficult to collect enough amounts of sera, and sometimes different patients had different reaction profiles to the certain allergenic proteins. We now are planning to combine cytokine (IL-4) stimulation and transformation of PBL by EBV followed by immediate cell fusion with mouse myeloma cells to establish mouse-human hybridomas secreting IgE antibodies continuously [6,13].

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