Allergenicity Forecast Using Immortalized Cells

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Abstract

Since it is not easy to prepare a standard sera library for allergenicity testing, we planned to develop a new system to evaluate the allergenicity of transgenic plants using the B-cell immortalizing technique. We have developed a method to establish human antibody secreting cells by transforming B-cells with the Epstein-Barr virus (EBV) followed by cell fusion with murine myeloma cells. In our preliminary experiment, we obtained 1,412 multi-clone immortalized human B-cell library stocks from seven healthy donors. The B-cell library contained several kinds of antibody-producing cells. The library stocks may contain antibodies to proteins we have never observed before. It is considered that our new technology will enable the evaluation of the allergenicity of novel proteins derived from less commonly allergenic food or non-food sources.

Introduction

Up to one-third of the population suffers from allergies. Allergies are mediated by antibodies of IgE class. This type of hypersensitivity is designated as type I hypersensitivity. When antigen (allergen) binds IgE molecules on the mast cell surface and cross-links two high affinity IgE receptors, the mast cell releases chemical mediators such as histamine and serotonin. Histamine causes smooth muscle contraction, increased vascular permeability and itching, and serotonin causes vasospasm and smooth muscle contraction.

Novel proteins synthesized in transgenic plants should be evaluated for their allergenicity. At the "3rd International Symposium on The Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms", Prof. Taylor discussed the evaluation of the allergenicity of foods developed through biotechnology (Taylor, 1995). He examined allergen immunoassays, skin testing, challenge testing, and comparisons to known allergens.

Since it is not easy to prepare a standard sera library for allergenicity testing, we planned to develop a new system to evaluate the allergenicity of transgenic plants employing the B-cell immortalizing technique. The specificity of antibody molecules can be defined by V-D-J (heavy chain) and V-J (light chain) rearrangement of the immunoglobulin gene (Okada and Alt, 1995). The possible number of specificities of antibodies was estimated to be more than 100,000,000. Recently, we developed a method to establish human antibody-secreting cells by transforming B-cells with the Epstein-Barr

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virus (EBV) followed by cell fusion with murine myeloma cells.

In this paper, we outline our approach to the analysis of the allergenicity of novel proteins in transgenic plants.

Strategies

We have just started the project for allergenicity forecasting of transgenic plants. The objectives of the project are to prepare an immortalized B-cell library as a source of antibodies and antibody-producing cells, and determine the *VH* gene usage to assess the allergenicity of novel proteins.

1. Immortalization of human B-cells

The Epstein-Barr virus (EBV) is a herpes virus that infects human B-cells (B-lymphocytes) and transforms them into immortalized B-lymphoblastoid cells (BLCs) (Shinmoto *et al.*, 1992). BLCs are known to secrete antibodies (immunoglobulins) . By using the EBV transformation method, we can obtain continuous antibody-producing BLCs. These are expected to include antibodies specific to allergens.

If one B-cell out of 1,000 peripheral blood lymphocytes (PBLs) is immortalized by EBV, 10 ml of blood sample may give 10,000 - 15,000 immortalized BLCs. We planned to immortalize more than 200,000 B-cell clones. As our "multiclone immortalized B-cell library stock" will be derived from 100,000 peripheral blood lymphocytes, it will contain 100 clones of immortalized BLCs.

We store the BLCs in a liquid nitrogen container and their supernatants at -80℃. The supernatants will be used for the analysis of antigen-specific antibodies.

2. Analysis of specific antibody-producing cell clones

Antibody-producing cells will be analyzed by an enzyme-linked immunosorbent assay (ELISA). Food allergens such as rice 16 kDa allergenic protein (Adachi *et al.*, 1995; Alvarez *et al.*, 1995) and Kunitz-type soybean trypsin inhibitor (Yamanishi *et al.*, 1995) will be used for antigens, and translation products from plasmids used for the generation of transgenic plants will be also used to analyze the allergenicity of those novel proteins. When a plasmid carries a kanamycin resistance gene, its translation product, aminoglycoside-3'-phosphotransferase II, will be used as an antigen (Motoyoshi, 1993).

We will then clone the antibody producing BLCs. Cloning of BLCs is very difficult, because the growth of BLCs is cell density-dependent. Usually BLCs cannot grow from single cells, even if irradiated feeder cells are added. To solve this problem, we will fuse BLCs with mouse myeloma cells. The resulting mouse-human hybridomas may easily be cloned (Shinmoto *et al.*, 1991a; 1991b).

There are reports on *VH* gene usage. Snow *et al.* (1995) reported the analysis of Ig *VH* region genes in splenic B-lymphocytes of a patient with asthma by PCR using

nested *VH*-Cε primer pairs. They showed the preferential usage of the *VH5* family derived from a single germ line gene *V5-51*. Wang *et al.* reported the preferential use of *VH3* in human anti-Gal heavy chain genes. Based on these reports, we consider that antibodies against allergens have their own rules of *VH* gene usage. If so, it must be possible to forecast allergenicity by analyzing the *VH* gene usage of BLCs secreting antibodies specific to novel proteins expressed in transgenic plants.

Preliminary experiment

Since we have not yet completely prepared the "multiclone immortalized B-cell library", we will describe the preliminary results of the preparation of another immortalized B-cell library .

1. Immortalization of PBLs with EBV

PBLs were prepared from seven healthy donors. B-cells were prepared from PBLs by the E rosette method using AET-treated sheep red blood cells. PBLs or B-cells infected with EBV were seeded into 96 well microculture plates. After 3 to 6 weeks of culture, wells with transformed lymphocytes were transferred to 24 well plates and cultured for 1 week. Cells were further cultured in a 6 cm dish, and cells (BLCs) and the supernatants were frozen (Fig. 1).

Table 1 shows the transformation of PBLs and / or B-cells from seven healthy donors with EBV (Shinmoto *et al.*, 1990). The transformation rate depended on the donor. Removal of T cells from PBLs obtained from those which yielded a low transformation rate resulted in a much higher transformation rate. We obtained a total of 1,412 multi-clone stocks of BLCs for the cell library.

2. ELISA of the culture supernatants

Culture supernatants of BLCs were assayed for antibodies to several antigens. As shown in Table 2, we detected 27 BLCs stocks containing BLCs secreting specific antibodies (IgA, IgG and IgM classes).

3. Cell fusion of BLCs with mouse myeloma SP2/O2

The 8-azaguanine resistant mouse myeloma cell line SP2/O was cultured with ouabain to obtain the ouabain-resistant cell line SP2/O2. BLCs specific to ricin and diphtheria toxin and SP2/O2 cells were fused and the cells were cultured in a HAT medium containing ouabain (Fig. 2). Table 3 shows the results (Shinmoto *et al.*, 1990; 1991a). The cell fusion efficiencies were high with all the clones. The clones listed in Table 3 were established after cloning. We also obtained hybridomas secreting antibodies specific to neocarzinostatin and cytomegalo virus (Shinmoto *et al.*, 1991b).

Conclusion

A cell library consisting of more than 1,000 BLCs stocks would be an excellent source of antibodies to a wide spectrum of antigens. In a preliminary experiment, we obtained an immortalized human B-cell library consisting of 1,412 multi-clone stocks without prior immunization. It contained antibodies to not only various viruses but also to ricin and neocarzinostatin. However, the disadvantages of this method lie in the instability of the clones secreting antibodies of interest and, thus, in the difficulty in establishing single clones. In contrast, cell fusion of human lymphocytes (non-transformed) with mouse myeloma yields high fusion efficiency and enables cloning by limiting dilution. However, it is difficult to establish hybridomas secreting antibodies with a certain specificity. The method described in this paper combined the advantages of both the cell library and cell fusion with mouse myeloma, and eliminated disadvantages.

The presence of clones secreting anti-neocarzinostatin antibodies suggested that library stocks may contain antibodies to novel proteins never observed before. It is considered that our new technology will enable to evaluate the allergenicity of novel proteins derived from less commonly allergenic food or non-food sources.

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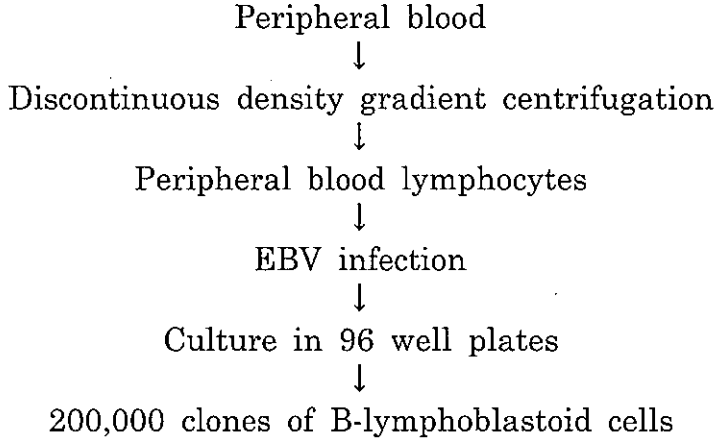

Fig. 1 Preparation of EBV immortalized cells

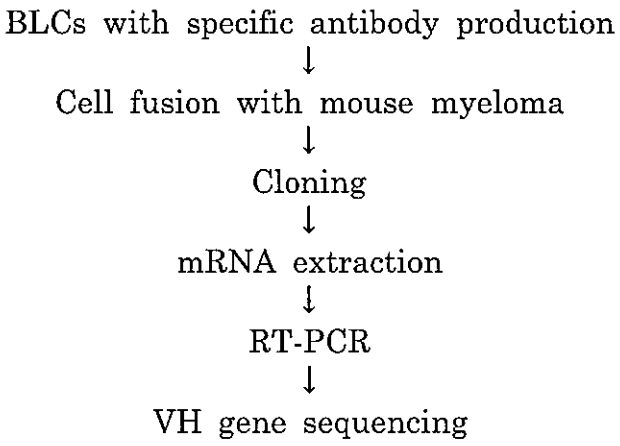

Fig. 2 Antibody VH gene usage analysis

Table 1 Transformation of PBLs and B-cells from normal donors with EBV

Donors		Cells (x 10^6)	Wells with transformed cells	Transformation rate*
HS	PBLs	54	536	9.9
MH	PBLs	34	280	8.2
HK	PBLs	10	92	9.2
YK	PBLs	18	207	11.5
SD	PBLs	26	15	0.6
	B-cells	1.8	60	3.3
MT	PBLs	10	16	1.6
	B-cells	4.5	71	15.8
KS	PBLs	13	11	0.8
	B-cells	4	124	31.0

*Wells with transformed cells / 10^6 cells.

Table 2 ELISA analysis of the supernatants of BLCs library stocks

Antigens	Positive stocks
Mycoplasma	2
Toxoplasma	3
Hepatitis B virus	2
Influenza virus	
A Yamagata	5
A Fukuoka	1
A Bangkok	0
B Ibaraki	2
Polio virus	
type 1	0
type 2	1
type 3	0
Adeno virus	0
Herpes simplex virus	1
Rota virus	1
Cytomegalo virus	3
Rubella virus	2
Ricin	7
Diphtheria toxin	5
Neocarzinostatin	1

Table 3 Generation of mouse-human hybridomas secreting human anti-ricin and anti-diphtheria toxin antibodies by cell fusion of BLCs with mouse myeloma SP2/O2

BLCs	Cells fused ($\times 10^7$)	Wells with growth	Wells with antibody	Hybridoma established
Anti-ricin				
KS18	3.5	790	31	KS18H2739
YK45	1.3	384	384	YK45H7-3.5
MH108	1.2	384	384	MH108H16-1.5
Anti-diphtheria toxin				
YK149	2.0	384	384	YK149H8-68
MH161	2.0	384	360	MH161H19-5