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Topical ocular treatment with monoclonal antibody Fab fragments targeting Japanese cedar pollen Cry j 1 inhibits Japanese cedar pollen-induced allergic conjunctivitis in mice

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ABSTRACT

Fab fragments (Fabs) of antibodies having the ability only to bind to specific allergens lack effector functions due to the absence of the Fc portion. In the present study, we examined whether IgG1 monoclonal antibody (mAb) Fabs targeting Japanese cedar pollen (JCP) Cry j 1 were able to regulate JCP-induced allergic conjunctivitis in mice. BALB/c mice actively sensitized with JCP were repeatedly challenged by topical administration of JCP eye drops. Fabs prepared by the digestion of anti-JCP IgG1 mAbs (P1-3 and P1-8) with papain were applied to the eye 15 min before the JCP challenges followed by measurement of the clinical conjunctivitis score. In the in vitro experiments, P1-3 and P1-8 showed specific binding to JCP Cry j 1. Furthermore, intact P1-3 binding to Cry j 1 was inhibited by P1-3 Fabs, but not P1-8 Fabs; additionally, P1-8 Fabs, but not P1-3 Fabs, suppressed the intact P1-8 binding, suggesting that the epitopes of Cry j 1 recognized by P1-3 and P1-8 were different. Topical ocular treatment with P1-3 Fabs or P1-8 Fabs was followed by marked suppression of JCP-induced conjunctivitis ($P < 0.01$). In histological evaluation, P1-8 Fabs showed a reduction in eosinophil infiltration in the conjunctiva ($P < 0.01$). These results demonstrated that topical ocular treatment with IgG1 mAb Fabs to Cry j 1 was effective in suppressing JCP-induced allergic conjunctivitis in mice. Furthermore, it suggests the possibility that some epitopes recognized by Fabs could be used as a tool to regulate allergic conjunctivitis.

1. Introduction

Allergic conjunctivitis is a type I allergic disease with a high prevalence (Friedlaender, 1991; Bielory and Friedlaender, 2008). The disease is characterized by early and late phase responses after exposure to the allergen. The early response involves itching, hyperemia, and oedema; the infiltration by inflammatory cells including eosinophils into the conjunctiva is recognized in the late phase (Hingorani et al., 1998). Furthermore, in the therapies for allergic conjunctivitis, not only several drugs including anti-histamines, vasoconstrictors, mast cell stabilizers, and glucocorticoids (Bisca, 1997), but also subcutaneous and sublingual allergen immunotherapies (Dretzke et al., 2013; Casale and Stokes, 2014) are used.

Fab fragments (Fabs) of antibodies maintain the ability to bind specific allergens although the lack of an effector function associated with the absence of Fc portion is recognized. Clinically, cetrtolizumab is a monoclonal antibody (mAb) Fab targeting TNF-α and used in the treatment of patients with rheumatoid arthritis or Chron's disease (Nesbitt et al., 2007; Deeks, 2013; Da et al., 2013). Furthermore, we have reported that the topical application of allergen-specific IgG1 mAb Fabs, but not the intact IgG1 mAb, suppressed allergen-induced asthma (Yoshino et al., 2014), rhinitis (Matsuoka et al., 2014), and dermatitis in mice (Sae-Wong et al., 2016). On the basis of the information, Fabs may be useful for the allergen-specific immunotherapy of allergic diseases; the treatment is expected to show immediate effects compared with subcutaneous and sublingual allergen immunotherapies, requiring long-term treatment (Abramson et al., 1995; Pipet et al., 2009; Keles et al., 2011; Passalacqua and Canonica, 2011). However, whether allergen-specific IgG1 mAb Fabs suppress allergen-induced allergic conjunctivitis in mice is unknown.

In the present study, we found that P1-3 and P1-8 showing Japanese cedar pollen (JCP) Cry j 1-specific IgG1 mAbs recognized different epitopes of Cry j 1. These data prompted us to investigate whether both Fabs suppress JCP-induced allergic conjunctivitis.

Abbreviations: H & E, hematoxylin and eosin; OVA, ovalbumin; JCP, Japanese cedar pollen; mAb, monoclonal antibody; P1-3 and P1-8, Cry j 1-specific IgG1 mAb

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Therefore, we examined the effects of topical ocular treatment with the two IgG1 mAb Fabs (P1-3 Fabs and P1-8 Fabs) on JCP-induced allergic conjunctivitis in mice.

2. Materials and methods

2.1. Animals

Male 7-week-old BALB/c mice were obtained from Japan SLC (Hamamatsu, Japan). These mice were maintained in a temperature-controlled environment with free access to standard rodent chow and water. All of the experimental procedures were approved by the Experimental Animal Research Committee at Kobe Pharmaceutical University.

2.2. Production and purification of anti-JCP IgG1 mAb (P1-3 and P1-8) and Fabs

Mice were sensitized with JCP (Biostir, Hyogo, Japan) emulsified with complete Freund's adjuvant (Sigma-Aldrich Fine Cheicals, MI, USA) and the spleen cells were fused with NS-1 myeloma cells, followed by screening and cloning hybridomas that produced anti-JCP mAbs, as previously described (Terato et al., 1992). Hybridomas producing anti-JCP IgG1mAbs including P1-3 and P1-8 were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 1% L-glutamine and 1% penicillin-streptomycin. To investigate the level of antibodies produced from the hybridomas (P1-3, P1-5, P1-6, P1-7, P1-8, and P1-9), the supernatants were incubated with JCP (100 μg/mL) coated on 96-well plates. After washing, this was followed by addition of an alkaline phosphatase-conjugated anti-Fc of mouse IgG1. The plate was developed with p-nitrophenyl phosphate, and measurements were made at 405 nm using a microplate enzyme-linked immunosorbent assay (ELISA) reader. As a result, the two hybridomas (P1-3 and P1-8) showed high absorbance compared with P1-5, P1-6, P1-7, and P1-9 (Table 1). Furthermore, P1-3 and P1-8 had the ability to specifically bind to IgG1 as shown by using a Mouse Monoclonal Sub-Isotyping Kit (American Qualex International Inc., La Mirada, CA, USA) (Table 2); therefore, we used two anti-JCP IgG1 mAbs (P1-3 and P1-8) for the in vivo experiments.

P1-3- and P1-8-producing hybridomas were grown in the CELLine CL1000 with a BD-Cell-Mab medium (BD Biosciences, San Diego, CA, USA) supplemented with 20% heat-inactivated FBS, 1% L-glutamine and 1% penicillin-streptomycin. To prepare P1-3 Fabs and P1-8 Fabs, the mAbs purified using a protein G column (GE Healthcare UK Ltd., Little Chalfont, UK) were digested with agarose-linked papain (Sigma-Aldrich, St. Louis, MO, USA) according to the methods described previously (Katpally et al., 2008). P1-3 Fabs and P1-8 Fabs were separated using a protein G column.

2.3. Allergenic specificity of P1-3 and P1-8

To investigate the allergenic specificity of P1-3 and P1-8, the mAbs (1 and 10 μg/mL) were incubated with 2 μg/mL of various allergens including Cry j 1, Cry j 2, ovalbumin (OVA), haemocyanin, collagen, and casein coated on 96-well plates. After washing, the alkaline phosphatase-conjugated anti-Fc of mouse IgG1 was added. Each plate was developed with p-nitrophenyl phosphate, and measurements were made at 405 nm using a microplate ELISA reader.

Table 1
The binding ability to Japanese cedar pollen in IgG1 mAbs produced by hybridomas.

	P1-3	P1-5	P1-6	P1-7	P1-8	P1-9
OD (405 nm)	1.907 ± 0.020	0.905 ± 0.016	0.283 ± 0.072	0.187 ± 0.009	1.692 ± 0.031	0.166 ± 0.056

The supernatants of cultured hybridomas were incubated with 100 μg/mL of JCP coated on the plate. This was followed by addition of alkaline phosphatase-conjugated anti-Fc of mouse IgG1. The plate was developed with p-nitrophenyl phosphate, and measurements were made at 405 nm using a microplate ELISA reader.

Table 2
The P1-3 and P1-8 ability to specifically bind to IgG1.

OD (405 nm)						
	IgG1	IgG2a	IgG2b	IgG3	IgM	IgA
P1-3	0.463 ± 0.042	0.012 ± 0.001	0.020 ± 0.008	0.007 ± 0.003	0.010 ± 0.003	0.009 ± 0.001
P1-8	0.450 ± 0.027	0.015 ± 0.004	0.007 ± 0.005	0.006 ± 0.003	0.005 ± 0.003	0.010 ± 0.002

The P1-3 and P1-8 ability to specifically bind to IgG1 was investigated by using a Mouse Monoclonal Sub-Isotyping Kit.

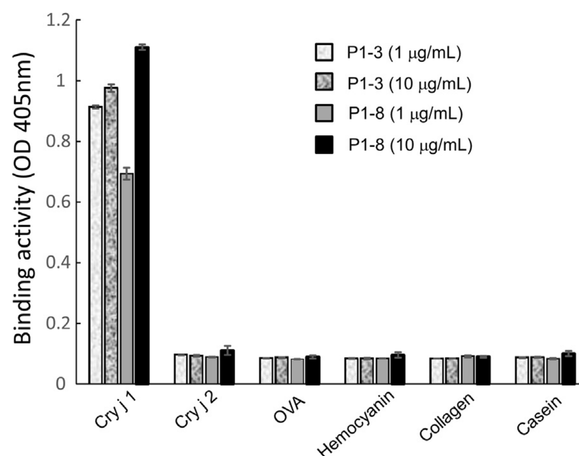

Fig. 1. P1-3 and P1-8 specifically bind to Cry j 1. The indicated amounts of P1-3 and P1-8 were added to various allergens including Cry j 1, Cry j 2, OVA, haemocyanin, collagen, and casein coated on the plate. This was followed by the further addition of an alkaline phosphatase-conjugated anti-Fc of mouse IgG1. Then, p-nitrophenyl phosphate was added to the plates before measuring absorbance at 405 nm using an ELISA reader.

2.4. Analysis of epitope specificity of P1-3 and P1-8

To investigate epitope specificities of P1-3 and P1-8, P1-3 and P1-8 Fabs (1–30 μg/mL) were incubated with Cry j 1 (2 μg/mL) coated 96-well plates. After washing, intact P1-3 or P1-8 (0.5 μg/mL) was added. To measure the intact P1-3 or P1-8 binding to Cry j 1, an alkaline phosphatase-conjugated anti-Fc of mouse IgG1 was added; the plate was developed with p-nitrophenyl phosphate, and measurements were made at 405 nm using a microplate ELISA reader. Additionally, P1-3 Fabs or P1-8 Fabs only were prepared as a negative control.

2.5. Induction of allergic conjunctivitis

As shown in Fig. 3, sensitization was performed by i.p. injection of JCP adsorbed to alum (WAKO Pure Chemical Industries, Ltd., Osaka, Japan) (100 μg JCP/5 mg alum/0.5 mL/mouse) on days 0 and 14. The sensitized mice were challenged by topical administration of eye drops of JCP (100 μg/4 μL/mouse) under anaesthesia with isoflurane (WAKO Pure Chemical Industries) on days 28, 29, 30, and 35. The occurrence of conjunctivitis was examined 10, 20, and 30 min after each antigen challenge. Four clinical signs were observed: conjunctival oedema; palpebral oedema; conjunctival hyperemia, and lacrimation. A scale of 0–4 with increments of 0.5 was used for each sign. Thus, each animal was given a total clinical score between 0 and 16.

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