

Brain-derived neurotrophic factor selectively enhances allergen-specific IgE production

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Abstract

We studied the effect of brain-derived neurotrophic factor (BDNF) on in vitro Japanese cedar pollen (JCP)-specific IgE production by mononuclear cells from atopic keratoconjunctivitis patients with JCP allergy. BDNF enhanced JCP-specific IgE production in a dose-dependent fashion in cultures of mononuclear cells stimulated with JCP, and maximal enhancement was achieved at 10 ng/ml. In contrast, BDNF had no effect on JCP-specific IgA or IgG4 production. On the other hand, other neurotrophins, NGF, NT-3, or NGF failed to enhance JCP-specific IgE production. Moreover, anti-BDNF mAb specifically blocked BDNF-induced enhancement of JCP-specific IgE production. Study for cytokine production revealed that BDNF decreased production of Th1 cytokines, IFN- γ and IL-12, while it had no effect on production of TH2 cytokines, IL-4, IL-10 and IL-13, in cultures of mononuclear cells stimulated with JCP. These results indicate that BDNF relatively skews cytokine pattern toward Th2 type. Collectively, BDNF may increase allergen-specific IgE production, which may in turn aggravate allergic symptoms.

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Keywords: Brain-derived neurotrophic factor; Japanese cedar pollen-specific IgE production; Atopic keratoconjunctivitis; Th1 and Th2 cytokine

1. Introduction

We have previously reported that neurotrophin, nerve growth factor (NGF) selectively enhances in vitro IgG4 production (Kimata et al., 1991). Patients with atopic dermatitis having keratoconjunctivitis (AKC) had elevated levels of allergen-specific IgG4 and IgE in tears (Kimata, 2004a). These patients also had elevated levels of NGF and another neurotrophin, brain-derived neurotrophic factor (BDNF) in tears (Kimata, 2004b).

On the other hand, BDNF receptor, trkB, was detectable in peripheral blood mononuclear cells, B cell lines, and Th1, but not Th2 or Th0 clones, in human (Besser and Wank, 1999). In another study, BDNF and trkB

were expressed in B cells of normal subjects (Edling et al., 2004). In addition, BDNF reduced serotonin uptake in human B cell lines (Mossner et al., 2000). These reports indicate that B cells may be regulated by BDNF. However, the effect of BDNF on immunoglobulin production by B cells was not reported.

Peripheral blood mononuclear cells (MNC) from AKC patients with Japanese cedar pollen (JCP) allergy produced JCP-specific IgE upon stimulation with JCP allergen (Kimata, 2003a,b). Using this in vitro system, the effect of BDNF on JCP-specific IgE production was studied.

2. Materials and methods

These studies were conducted with the approval of the Ethical Committee at the Satou Hospital.

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2.1. Subjects

After obtaining informed consents, peripheral blood was sampled from 15 AKC patients with JCP allergy. All of the patients had mild atopic dermatitis (SCORAD index < 15) (Kunz et al., 1997), and were allergic to JCP as determined by positive skin prick test to JCP and positive serum JCP-specific IgE (Kimata, 2003a). Moreover, all of the patients were allergic to house dust mite (HDM) as determined by positive skin prick test and positive serum HDM-specific IgE, while none of them had allergy to latex. Mean serum JCP-specific IgE and HDM-specific was 57.3 IU/ml and 45.1 IU/ml, respectively, while serum latex-specific IgE was not detected (<0.34 IU/ml). All 15 of AKC patients were treated with application of mixture of pinetar (Yoshida Pharmaceutical Co., Ltd., Tokyo, Japan) and zinc oxide ointment for skin, and tranilast eyedrops (Kissei Pharmaceuticals, Co., Ltd., Tokyo, Japan) for eye, but without oral medication (Kimata, 2004a). All of the patients did not apply these medication 2 days before study.

2.2. Cell cultures

Mononuclear cells (MNC) from blood were separated, and they were cultured (2×10^4 /0.2 ml/well) with 0.1 ng/ml of JCP (Torii Pharmaceuticals, Co. Ltd., Tokyo, Japan) or 0.1 ng/ml of latex (C-serum) (Kimata, 2003a). Cultures were stimulated with different concentrations of human recombinant BDNF, NT-3, NT-4, or NGF with or without anti-BDNF mAb or anti-NGF mAb (Promega, Madison, MI). In some experiments, IFN- γ (10 U/ml) and/or IL-12 (10 ng/ml) were added to BDNF. After 14 days of culture, production of JCP- or latex-specific IgE, IgA1 and IgG4 was determined by ELISA (Kimata, 2003a), while after 5 days of culture, production of IL-4, IL-10, IL-12, IL-13 and IFN- γ was determined by ELISA (R&D Systems, Inc. Minneapolis, MN). The culture medium was serum-free medium, DME, supplemented with Ham's Nutrient (DME/F-12; Sigma Chemical Co. (St. Louis, MO), 0.5% BSA, and 50 μ g/ml transferrin (Kimata et al., 1996).

2.3. Tear collection and measurement of JCP-specific IgE

Tears were collected with a micropipette (Drummond Scientific Broomall, PA) as previously reported (Yamada et al., 2002). BDNF and JCP-specific IgE in tears were measured by ELISA (Promega) and by our assay, respectively (Kimata, 2004a,b). These studies were conducted with the approval of the Ethical Committee at the Satou Hospital.

2.4. Statistical analysis

Unless specified, statistical analysis was performed with ANOVA with a multiple comparison of mean test.

Spearman rank-order correlation coefficient was used to compare BDNF levels and JCP-specific IgE levels in tear.

3. Results

3.1. Effect of BDNF on JCP-specific immunoglobulin production

In preliminary experiments, we studied the effect of BDNF on JCP-specific IgE production on healthy non-atopic individuals ($n=20$) or AKC patients without JCP allergy ($n=18$). However, MNC from these subjects failed to produce JCP-specific IgE (<0.15 ng/ml) upon JCP stimulation with or without BDNF.

In contrast, MNC from AKC patients with JCP allergy produced significant amount of JCP-specific IgE upon JCP stimulation (Fig. 1). BDNF enhanced JCP-specific IgE production in a dose-dependent fashion in JCP-stimulated cultures, and maximal enhancement was achieved at 10 ng/ml of BDNF. In contrast,

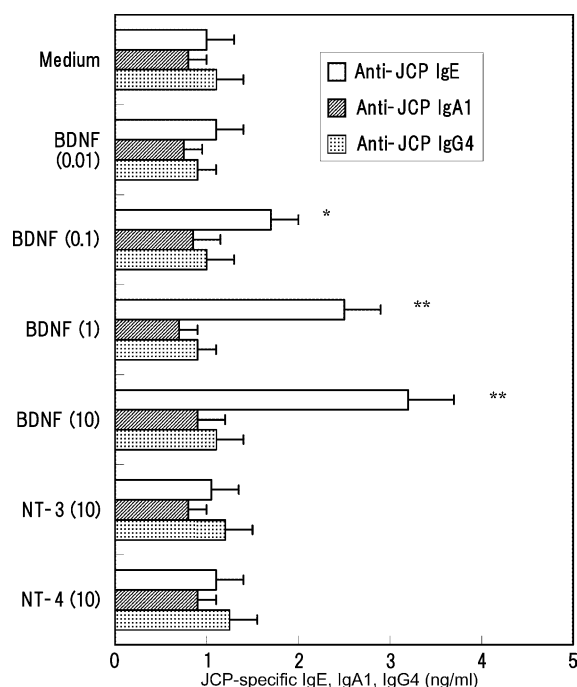


Fig. 1. Effect of BDNF on JCP-specific IgE, IgA1 and IgG4 production by mononuclear cells from AKC patients with JCP allergy. Mononuclear cells from AKC patients with latex allergy were stimulated with JCP (0.1 ng/ml) with or without indicated factors, and JCP-specific IgE, IgA1 and IgG4 production was measured. BDNF was used from 0.01, 0.1, 1 or 10 ng/ml, as indicated as BDNF (0.01), BDNF (0.1), BDNF (1), or BDNF (10). NT-3 and NT-4 were used at 10 ng/ml as NT-3 (10) and NT-4 (10), respectively. Values are the means $\pm$ SD from four experiments. * Significant enhancement ($p < 0.05$) compared to cultures with medium. ** Significant enhancement ($p < 0.01$) compared to cultures with medium.

BDNF failed to enhance production of JCP-specific IgA1 or IgG4 (Fig. 1). Although not shown, JCP-stimulated MNC failed to produce JCP-specific IgG1, IgG2, IgG3, IgM or IgA2, and addition of BDNF at concentrations from 0.01 to 10 ng/ml failed to induce them. Effect of the other neurotrophins, NT-3 and NT-4, was also studied. However, neither NT-3 nor NT-4 at 10 ng/ml enhanced JCP-specific IgE, IgA1 or IgG4 production (Fig. 1). Moreover, NT-3 or NT-4 at concentrations from 0.01 to 100 ng/ml failed to enhance JCP-specific IgE, IgA1 or IgG4 production (data not shown).

The effect of pro IgE cytokine, IL-4, was also tested in this assay. The level of JCP-specific IgE production (mean + SD ng/ml) by IL-4 at 1, 10, and 100 ng/ml were induced 2.9 + 0.6, 4.7 + 0.9 and 6.8 + 1.3, respectively.

3.2. Specificity of the effect of BDNF

The specificity of the effect of BDNF was then studied. We have previously reported that NGF enhanced IgG4, but not IgE, production (Kimata et al., 1991a). Thus, effect of BDNF was compared to the effect of NGF. As shown in Table 1, BDNF-induced enhancement of JCP-specific IgE production was blocked by anti-BDNF mAb, but not by anti-NGF mAb. In contrast, NGF-induced enhancement of IgG4 production was blocked by anti-NGF mAb, but not by anti-BDNF mAb. Control mAb failed to block BDNF-induced enhancement of IgE production or NGF-induced enhancement of IgG4 production (data not shown).

3.3. Allergen specificity of enhancement of IgE production by BDNF

Allergen specificity was then studied. As shown in Table 2, BDNF significantly enhanced JCP-specific IgE production when the culture was stimulated by JCP, but not by HDM or latex. On the other hand, BDNF enhanced HDM-specific IgE production when the culture was stimulated by HDM, but not by JCP or latex.

In contrast, since none of patients were allergic to latex, no latex-specific IgE was produced by latex-stimulated MNC, and BDNF failed to induce latex-specific IgE production.

3.4. Effect of BDNF on cytokine production

Allergen-specific IgE production was increased by Th2 cytokines, IL-4, IL-10 and IL-13, while it was decreased by Th1 cytokines, IFN- γ and IL-12 as previously reported (Kimata, 2003a,b). Therefore, the effect of BDNF on JCP-induced cytokine production was studied. As shown in Table 3, BDNF decreased production of Th1 cytokines, IFN- γ and IL-12, while BDNF had no effect on production of Th2 cytokines, IL-4, IL-10 and IL-13 in 3 representative patients. Similar results were observed in other 12 patients (data not shown). These results indicate that BDNF skewed cytokine pattern toward Th2 type.

Table 1
Specificity of the BDNF-induced enhancement of JCP-specific IgE production

Factors	JCP-specific Ig production (pg/ml)			
	Experiment 1		Experiment 2	
	IgE	IgG4	IgE	IgG4
Medium	1.3 (0.3)	0.9 (0.2)	2.6 (0.5)	1.4 (0.3)
BDNF	3.5 (0.5)*	0.8 (0.2)	5.1 (0.7)*	1.2 (0.3)
BDNF + anti-BDNF mAb	1.4 (0.3)	0.8 (0.2)	2.7 (0.5)	1.5 (0.4)
BDNF + anti-NGF mAb	3.4 (0.5)*	0.7 (0.2)	5.0 (0.7)*	1.3 (0.3)
NGF	1.5 (0.3)	3.2 (0.5)*	2.7 (0.5)	3.7 (0.7)*
NGF + anti-BDNF mAb	1.4 (0.3)	3.0 (0.5)*	2.8 (0.6)	3.5 (0.7)*
NGF + anti-NGF mAb	1.2 (0.3)	0.9 (0.2)	2.5 (0.5)	1.5 (0.3)

MNC from AKC patients with JCP allergy were cultured with JCP (0.1 ng/ml) with medium or indicated factors, and JCP-specific IgE and IgG4 production were measured. BDNF and NGF were used at 10 ng/ml, while anti-BDNF mAb and anti-NGF mAb were used at 1 mg/ml. Values are the means (SD) of quadruplicate cultures in two experiments out of five experiments. Although not shown, similar results were obtained in other three experiments.

* Significant decrease ($p < 0.01$) compared to cultures with medium.

Table 2
Allergen specificity of the BDNF-induced enhancement of IgE production

Allergen	Allergen-specific IgE production (ng/ml)					
	JCP-specific IgE		HDM-specific IgE		Latex-specific IgE	
	Medium	BDNF	Medium	BDNF	Medium	BDNF
None	<0.34	<0.34	<0.34	<0.34	<0.34	<0.34
JCP	1.7 (0.3)	3.8 (0.4)*	<0.34	<0.34	<0.34	<0.34
HDM	<0.34	<0.34	2.0 (0.4)	4.5 (0.6)*	<0.34	<0.34
Latex	<0.34	<0.34	<0.34	<0.34	<0.34	<0.34

MNC from AKC patients with JCP allergy were cultured with JCP (0.1 ng/ml) with medium or indicated factors, and JCP-specific IgE and IgG4 production were measured. BDNF and NGF were used at 10 ng/ml, while anti-BDNF mAb and anti-NGF mAb were used at 1 mg/ml. Values are the means (SEM) of 15 patients.

* Significant decrease ($p < 0.01$) compared to cultures with medium.

Table 3
Effect of BDNF on JCP-induced cytokine production

Cytokine	Cytokine production (pg/ml)					
	Patient 1		Patient 2		Patient 3	
	Medium	BDNF	Medium	BDNF	Medium	BDNF
IFN- γ	212 (35)	127 (29)*	145 (21)	87 (15)*	329 (68)	204 (57)*
IL-12	117 (27)	81 (16)*	156 (34)	107 (22)*	275 (55)	159 (40)*
IL-4	435 (69)	429 (68)	509 (85)	517 (90)	383 (72)	379 (70)
IL-10	213 (36)	206 (33)	347 (67)	358 (69)	168 (43)	154 (45)
IL-13	319 (46)	328 (53)	179 (30)	184 (34)	389 (73)	378 (64)

MNC from AKC patients with JCP allergy were cultured with JCP (0.1 ng/ml) with medium or BDNF (10 ng/ml), and cytokine production was measured. Values are the means (SD) of quadruplicate cultures in 3 patients. Although not shown, similar results were obtained in other 12 patients.

* Significant decrease ($p < 0.01$) compared to cultures with medium.

3.5. Blocking of the BDNF-induced enhancement of JCP-specific IgE production by IFN- γ and/or IL-12

To prove directly that the BDNF-induced enhancement of JCP-specific IgE production was decreased in IFN- γ and/or IL-12 production, MNC were cultured with JCP and BDNF, and IFN- γ and/or IL-12 were added. As shown in Table 4, addition of IFN- γ or IL-12 partially blocked BDNF-induced enhancement of JCP-specific IgE production. Moreover, addition of both IFN- γ and IL-12 completely blocked enhancement.

3.6. Correlation of BDNF levels and JCP-specific IgE levels in tear

Finally, levels of JCP-specific IgE and BDNF in tears of 15 AKC patients were measured and compared. As shown in Fig. 2, there was a significant correlation ($r_s = 0.87471$ by Spearman rank-order correlation coefficient, $p < 0.01$) between tear JCP-specific IgE levels and tear BDNF levels. The levels (mean $\pm$ SEM) of JCP-specific IgE and BDNF were 8.4 ± 0.3 ng/ml and 183 ± 4 pg/ml, respectively.

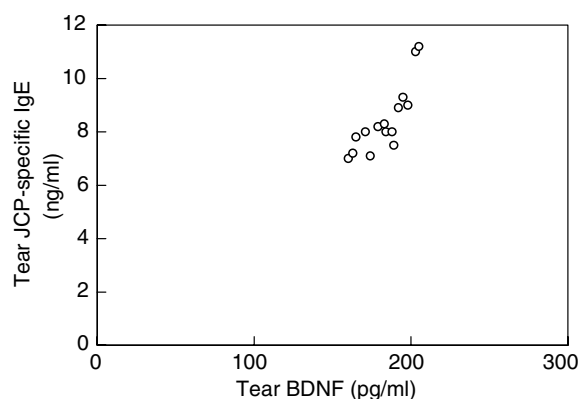


Fig. 2. Correlation of BDNF and JCP-specific IgE levels in tear of AKC patients. Tear levels of BDNF (X-axis) and JCP-specific IgE (Y-axis) were measured and plotted. Each point indicates value in each patient. $r_s = 0.87471$ by Spearman rank-order correlation coefficient, $p < 0.01$ between JCP-specific IgE and BDNF levels.

The levels of JCP-specific IgE and BDNF were also measured in tears of 16 healthy controls. No JCP-specific IgE was detected (< 0.15 ng/ml), and BDNF (mean $\pm$ SEM) levels were low (91 ± 3 pg/ml) compared to those in AKC patients with JCP allergy.

Table 4
Blocking of BDNF-induced enhancement of JCP-specific IgE production

Factors	JCP-specific IgE production (ng/ml)		
	Patient 1	Patient 2	Patient 3
Medium	1.5 (0.3)	1.0 (0.2)	0.8 (0.2)
BDNF	3.4 (0.5)*	3.8 (0.5)*	2.2 (0.4)*
BDNF + IFN- γ	2.8 (0.4)**	1.9 (0.4)**	1.4 (0.3)**
BDNF + IL-12	2.4 (0.3)**	2.5 (0.3)**	1.7 (0.3)**
BDNF + IFN- γ + IL-12	1.6 (0.3)	1.2 (0.2)	0.7 (0.2)

MNC from AKC patients with JCP allergy were cultured with JCP (0.1 ng/ml) with indicated factors. BDNF was used at 10 ng/ml, IFN- γ at 10 U/ml and IL-12 at 10 ng/ml. After 14 days of culture, JCP-specific IgE production was measured. Values are the means (SD) of quadruplicate cultures in 3 patients.

* Significant increase ($p < 0.01$) compared to cultures with medium.

** Significant increase ($p < 0.05$) compared to cultures with medium.

4. Discussion

These results demonstrated that BDNF enhanced JCP-specific IgE production without affecting JCP-specific IgA1 or IgG4 production in AKC patients with JCP allergy. This was specific to BDNF, since NT-3, NT-4 or NGF failed to enhance JCP-specific IgE production. In addition, BDNF-induced enhancement of IgE production was blocked by anti-BDNF mAb, but not by anti-NGF mAb or control mAb. Moreover, enhancement was specific to allergen to which patients were allergic, since BDNF enhanced HDM-specific IgE production in AKC patients who were allergic to HDM, while BDNF failed to induce latex-specific IgE production in AKC patients who were not allergic to latex.

It should be noted that BDNF decreased JCP-induced Th1 cytokine production (IFN- γ and IL-12) without affecting production of Th2 cytokines (IL-4, IL-10 and IL-13). Consequently, cytokine balance was skewed toward Th2 type, which correlated with enhanced JCP-specific IgE production. The fact that IgG4 production was not enhanced, was not surprising. We and others have previously reported that skewing cytokine toward Th2 type differentially regulate IgE, IgG subclass or IgA subclass production (Akdis et al., 1998; Carballido et al., 1994; Kimata et al., 1991). Detailed mechanisms of selective enhancement of JCP-specific IgE production are currently under investigation. However, it has been reported that full length mRNA of BDNF receptors were selectively detected in human Th1 cells and some portion of B cells (Besser and Wank, 1999). In accordance with this, our preliminary experiments ($n=4$) have shown that JCP-stimulated Th1 cells (CD4 + CXCR3 + T cells) expressed BDNF receptors (Δ mean fluorescence intensity: 35 ± 3), while JCP-stimulated Th2 cells (CD4CCR3 + T cells) failed to do so (Δ mean fluorescence intensity: <3). On the other hand, JCP-stimulated surface IgE + B cells (sIgE + B cells) expressed BDNF receptors (Δ mean fluorescence intensity: 26 ± 2), while JCP-stimulated sIgG4 + B cells or sIgA1 + B cells failed to do so (Δ mean fluorescence intensity: <0.3) (manuscript in preparation). Thus, BDNF selectively stimulated Th1 cells and sIgE + B cells through BDNF receptors.

The biological significance of BDNF on enhancement of IgE production remains to be elucidated.

However, we have previously reported that BDNF levels in tears are significantly elevated in AKC patients, while levels of NT-3 or NT-4 in tears are not so (Kimata, 2004a,b). Moreover, we have now demonstrated that levels of BDNF and JCP-specific IgE in tears are significantly correlated. Allergic responses were initiated by the binding of IgE to IgE receptor (Fc ϵ RI) and decrease in IgE concentrations by anti-IgE antibody has been proved to be effective in the treatment of allergic diseases (Felix Toledo et al., 2004; MacGlashan et al., 1997). It would be tempting to speculate BDNF may enhance allergen-specific IgE production, which in turn may augment allergic responses and aggravate allergic symptoms. This possibility is currently under investigation.

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