

Ciliary neurotrophic factor preferentially enhances spontaneous IgE production by B cells from atopic patients

H. Kimata *

Department of Allergy, Ujitakeda Hospital, 24-1, Umonji, Uji, Kyoto Prefecture 6110021, Japan

Received 24 February 2004; accepted 17 April 2004

Abstract

The effect of ciliary neurotrophic factor (CNTF) on IgE production by purified B cells from atopic patients was studied. CNTF significantly enhanced spontaneous IgE production by B cells from patients with atopic eczema/dermatitis syndrome (AEDS) in a dose-dependent fashion, and maximum enhancement was achieved at 1 ng/ml. CNTF-induced enhancement of IgE production was blocked by anti-CNTF mAb or anti-gp 130 mAb, but not by anti-IL-6 mAb. On the other hand, CNTF did not significantly enhance spontaneous production of IgG1, IgG2, IgG3, IgG4, IgM, IgA1 or IgA2 by B cells from AEDS patients. In contrast to B cells from AEDS patients, B cells from non-atopic subjects failed to produce IgE spontaneously, and CNTF did not induce IgE production by non-atopic subjects' B cells. B cells from atopic patients contained surface IgE positive B cells (sIgE+ B cells), which spontaneously produced IgE, while surface IgE negative B cells (sIgE– B cells) failed to do so. CNTF enhanced IgE production by sIgE+ B cells from patients with AEDS, allergic rhinitis or bronchial asthma, while CNTF failed to induce IgE from sIgE– B cells from these patients. Stimulation of sIgE– B cells with IL-4 plus anti-CD40 mAb induced IgE production. However, stimulation of sIgE– B cells with CNTF plus IL-4, or CNTF plus anti-CD40 mAb did not induce IgE production by sIgE– B cells. Collectively, these results indicate that CNTF preferentially enhanced spontaneous IgE production by post-switched sIgE+ B cells, while CNTF failed to induce IgE by pre-switched sIgE– B cells. These results suggest that CNTF may be involved in the allergic diseases.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Ciliary neurotrophic factor; Spontaneous IgE production; Atopic eczema/dermatitis syndrome; Bronchial asthma; Allergic rhinitis; Surface IgE positive and negative B cells

1. Introduction

B cells from atopic patients contained post-switched surface IgE positive B cells (sIgE+ B cells), which spontaneously produced IgE upon in vitro culture. In contrast, most B cells from normal subjects were pre-switched surface IgE negative B cells (sIgE– B cells), which did not produce IgE spontaneously upon in vitro culture (Kimata et al., 1996). Spontaneous IgE production was inhibited by neuropeptides (vasoactive intestinal peptide or somatostatin), high dose γ -globulin, or cytokines (IFN- γ or IL-8), while it was enhanced by histamine, hydrocortisone or IL-6 (Boguniewicz et al., 1990; Kimata et al., 1993; Kimata, 1995; Kimata et al., 1995; Kimata et al., 1996). These results have important

implication in the study and treatment of allergic diseases. In fact, in vivo administration of high dose γ -globulin or IFN- γ decreased serum IgE levels and improved symptoms of atopic eczema/dermatitis syndrome (AEDS) (Boguniewicz et al., 1990; Kimata, 1995).

Ciliary neurotrophic factor (CNTF) is a member of IL-6 cytokine family, and it promotes the maintenance, differentiation, and/or survival of a variety of neurons within the nervous system (Sendtner et al., 1994). In addition to the effect on nervous system, CNTF also acts on immune system. CNTF suppressed synthesis of IL-1 and prostaglandin E2 by human peripheral blood mononuclear cells (Shapiro et al., 1994), while CNTF enhances proliferation of human myeloma cell lines in an IL-6-independent fashion (Gu et al., 1996). Moreover, CNTF combined with soluble CNTF receptor α promotes mouse macrophage chemotaxis (Kobayashi and Mizisin, 2000). This is not surprising, since IL-6 fused to its soluble receptor

* Tel.: +81-774-25-2500; fax: +81-774-25-2353.

E-mail address: h-kimata@takedahp.or.jp (H. Kimata).

enhanced differentiation of rat oligodendrocyte progenitor cells (Valerio et al., 2002). These reports prompted us to study the effect of CNTF on spontaneous IgE production by B cells from atopic patients.

2. Materials and methods

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

2.1. Reagents

Recombinant human ciliary neurotrophic factor (CNTF), mouse IgG2a anti-CNTF mAb, mouse IgG1 anti-human gp130 mAb, and mouse IgG1 anti-IL-6 mAb were purchased from R & D Systems, Inc. (Minneapolis, MN). Recombinant human IL-4 was provided by Ono Pharmaceutical Company (Osaka, Japan). Mouse IgM anti-CD40 mAb and was purchased from Cosmo Bio Co. (Tokyo, Japan). Mouse anti-CD38 mAb (Becton–Dickinson, Mountain View, CA) and fluorescein-conjugated goat F(ab')₂ anti-mouse immunoglobulin (Tago Immunologicals, Burlingame, CA) were used to analyze the percentage of CD 38+ cells by a FACSCAN flow cytometer (Becton–Dickinson, Mountain View, CA) (Sherr et al., 1989). The culture medium was DME, supplemented with Ham's Nutrient (DME/F-12; Sigma Chemical Co. (St. Louis, MO), 0.5% BSA, and 50 µg/ml transferrin (Kimata et al., 1995).

2.2. Cell cultures

After obtaining informed consent, human tonsils from non-atopic subjects (serum IgE levels < 50 IU/ml), or patients with AEDS (serum IgE levels 1897–12765 IU/ml), allergic rhinitis (serum IgE levels 248–2859 IU/ml) or bronchial asthma (serum IgE levels 2839–5738 IU/ml) were obtained at tonsillectomy for chronic tonsillitis. Thereafter, tonsillar mononuclear cells separated, and B cells were highly purified by sheep red blood cell resetting, followed by L-leucine methyl ester incubation methods as reported previously (Kimata et al., 1995). Purified B cell fractions contained <1% CD3+ T cells, <1% CD14+ monocytes, <1% CD16+ NK cells, and >98% CD20+ B cells. Purified B cells from atopic patients were further separated into surface IgE positive (sIgE+) B cells or surface IgE negative (sIgE-) B cells by panning as previously reported (Kimata et al., 1995). The percentage of sIgE+ B cells in sIgE+ B cells and sIgE- B cells were >98% and <0.1%, respectively. Similarly, sIgG1+ B cells or sIgA1+ B cells were purified by panning as previously reported (Kimata et al., 1996). Whole B cells ($1 \times 10^5/0.2$ ml/well), or sIg+ or Ig- B cell populations ($2 \times 10^4/0.2$ ml/well) were cultured for 6 days in U-bottomed plates (Costar Corp., Cambridge, MA) in the presence or ab-

sence of various factors as described in Section 3. The amounts of IgE, IgG1, IgG2, IgG3, IgG4, IgM, IgA1 and IgA2 in the supernatants were determined by ELISA as previously reported (Kimata et al., 1996).

In some experiments, cell growth was measured by colorimetric assay using a test wavelength of 570 nm and a reference wavelength of 630 nm (OD₅₇₀₋₆₃₀) (Mossman, 1983). This assay is based on the fact that living cells utilizes a colorless substrate that is modified to a colored product by any living cell, but not by dead cells or tissue culture medium. Tetrazolium salts are suitable for this purpose, since they measure the activity of various dehydrogenase enzymes. The tetrazolium ring is cleaved in active mitochondria, and thus the reaction occurs only in living cells. Thus, tetrazolium salt MTT (3-(4,5-dimethylisothiazol-2-yl)-2,5-diphenyl tetrazolium bromide), that measured only living cells and can be read by ELISA reader, was used for this assay. Briefly, MTT (Sigma) was dissolved in PBS at 5 mg/ml, and stock solution (10 µl per 100 µl medium) was added to wells, and plates were incubated at 37 °C for 4 h. Acid-isopropanol was added to all wells, and after a few minutes at room temperature, the plates were read on ELISA reader using a test wavelength of 570 nm, a reference wavelength of 630 nm, and a calibration setting of 1.99. This assay measured the viable number of cells, and correlated with thymidine uptake by mitogen-stimulated cells (Mossman, 1983).

2.3. ELISA for IgE, IgG subclasses, IgA subclasses and IgA

Immunoplates (Nunc, Roskilde, Denmark) were coated with mixtures of 1 µg/ml of mouse IgG1 anti-IgE mAbs (CIA-7.12 and CIA-4.15) (provided Dr. Andrew Saxon, UCLA), 1 µg/ml of each of mouse IgG1 anti-human IgG1 mAb (ML-16), mouse IgG1 anti-human IgG2 mAb (HG2-56F), mouse IgG1 anti-human IgG3 mAb (HG3-7C), mouse IgG2b anti-human IgG4 mAb (HG4-53G), mouse anti-human IgG1 anti-human IgA1 mAb (1-155-1), mouse IgG2b anti-human IgA2 mAb (14-3-26), or goat anti-IgM Ab (200 µg/ml) at 4 °C overnight. The immunoplates were then washed with PBS–Tween, and samples or a standard serum were added. They were incubated overnight at 4 °C. Thereafter, peroxidase-conjugated goat anti-human Ig was added. After 1 h of incubation at room temperature, the reaction was stopped and absorbance was measured by ELISA reader. The sensitivities of the assays were 0.15 ng/ml for all classes Ig (Kimata et al., 1996).

2.4. Data analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using ANOVA with a multiple comparison of means test.

3. Results

3.1. Effect of CNTF on IgE production by B cells from non-atopic subjects or patients with AEDS

As shown in Fig. 1, B cells from non-atopic subjects did not produce IgE. CNTF at concentrations from 0.01 to 10 ng/ml failed to induce IgE by B cells from non-atopic subjects. In contrast, B cells from AEDS patients spontaneously produced IgE. CNTF enhanced the IgE production in a dose-dependent fashion, and maximal enhancement was achieved at 1 ng/ml of CNTF. This enhancement was specific to CNTF, since anti-CNTF mAb or anti-gp 130 mAb (mAb to IL-6 family receptor) blocked CNTF-induced enhancement of IgE production, while anti-IL-6 mAb or control mouse IgG1 failed to do so.

IL-6 enhanced spontaneous IgE production by B cells from atopic patients, while it failed to induce IgE production by B cells from non-atopic subjects (Kimata et al., 1995). However, as shown in Table 1, IL-6-induced enhancement of IgE production was blocked by anti-IL-6 mAb or anti-gp 130 mAb, but not by anti-CNTF mAb. These results indicate that CNTF-induced enhancement of IgE production is not mediated by IL-6.

3.2. Effect of CNTF on production of IgG subclasses, IgM and IgA subclasses

The effect of CNTF on other Ig classes was then studied. As shown in Table 1, B cells from non-atopic

Table 1
Effect of CNTF on various classes of Ig production by B cells

Ig class	Non-atopic subjects		AEDS patients	
	Medium	CNTF	Medium	CNTF
IgE	<0.15	<0.15	4.5 (0.7)	9.4 (1.3)*
IgG1	53 (5)	61 (6)	57 (5)	66 (6)
IgG2	36 (3)	39 (4)	42 (5)	37 (4)
IgG3	14 (2)	11 (3)	15 (2)	18 (2)
IgG4	1.2 (0.4)	1.4 (0.5)	3.8 (0.7)	3.5 (0.6)
IgM	60 (6)	72 (7)	89 (8)	93 (9)
IgA1	12 (4)	15 (4)	18 (4)	20 (4)
IgA2	2.4 (0.4)	2.1 (0.5)	4.1 (0.7)	3.8 (0.6)

B cells from non-atopic subjects or patients with AEDS (atopic eczema/dermatitis syndrome) were cultured with medium or CNTF (1 ng/ml), and various classes of Ig production were measured.

Values are the means (SEM) from six experiments.

* Significant enhancement ($P < 0.01$) compared to cultures with medium.

subjects spontaneously produced IgG1, IgG2, IgG3, IgG4, IgM, IgA1 and IgA2, but not IgE. CNTF had no effect on production of these Ig. On the other hand, B cells from AEDS patients produced all classes of Ig. While CNTF significantly enhanced IgE production, CNTF did not significantly enhance other class of Ig production. Thus, CNTF preferentially enhanced spontaneous IgE production.

3.3. Effect of CNTF on IgE production by sIgE- or sIgE+ B cells from atopic patients

B cells from atopic patients contained post-switched sIgE+ B cells, which spontaneously produced IgE

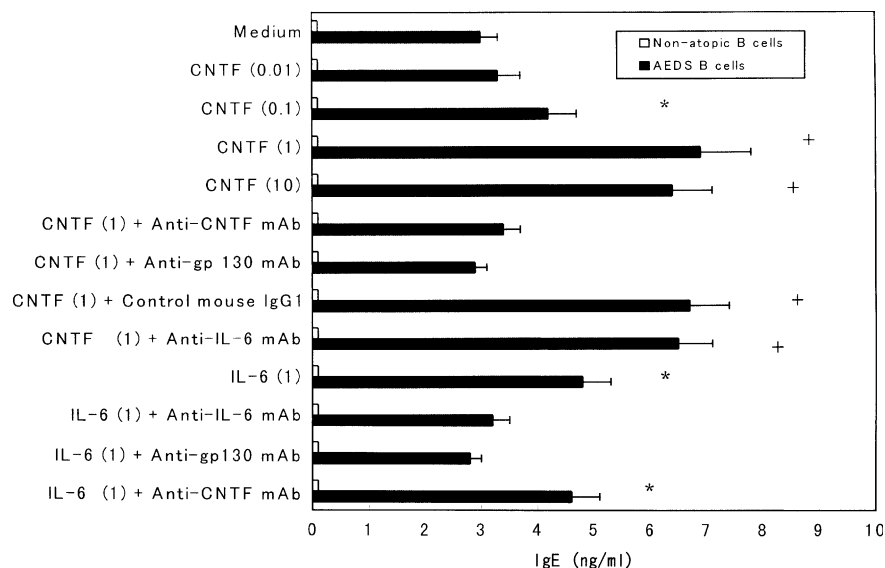


Fig. 1. Effect of CNTF on IgE production by B cells from non-atopic subjects or AEDS patients. B cells from non-atopic subjects or AEDS (atopic eczema/dermatitis syndrome) patients were cultured with indicated factors, and IgE production was measured. CNTF were used from 0.01, 0.1, 1 or 10 ng/ml, as indicated as CNTF (0.01), CNTF (0.1), CNTF (1), or CNTF (10). Anti-CNTF mAb, anti-gp 130 mAb, control mouse IgG1, and anti-IL-6 mAb were used at 5 μ g/ml. IL-6 was used at 1 ng/ml, as indicated as IL-6 (1). Values are the means $\pm$ SEM from six experiments. Non-atopic B cells with or without various factors failed to produce IgE <0.15 ng/ml. * Significant enhancement ($P < 0.05$) compared to cultures with medium. + Significant enhancement ($P < 0.01$) compared to cultures with medium.

upon in vitro culture, while pre-switched sIgE[−] B cells failed to do so. However, stimulation of IL-4 plus anti-CD40 mAb induced IgE production by sIgE[−] B cells, while IL-4 plus anti-CD40 mAb had no effect on IgE production by sIgE⁺ B cells (Kimata et al., 1996). Thus effect of CNTF on sIgE⁺ and sIgE[−] B cells was studied.

Table 2 shows that CNTF enhanced IgE production by sIgE⁺ B cells from atopic patients, including patients with AEDS, allergic rhinitis or bronchial asthma. Although IL-4 plus anti-CD40 mAb induced IgE production by sIgE[−] B cells from these patients, neither CNTF plus IL-4 nor CNTF plus anti-CD40 mAb induced IgE production by sIgE[−] B cells. Moreover, addition of IL-4 or anti-CD40 mAb to CNTF did not augment CNTF-induced enhancement of IgE production by sIgE⁺ B cells. Thus, CNTF acts on sIgE⁺ B cells as enhancing factor, but not acts as switching factor on sIgE[−] B cells.

3.4. Effect of CNTF on various classes of Ig production by sIg[−] or sIg⁺ B cells from atopic patients

The preferential effect of CNTF on IgE production may be due to differential effects of the CNTF on IgE-producing B cells and other Ig-producing B cells. It has been reported that most of IgE-producing sIgE⁺ B cells in atopic patients are plasma cells, while most of IgG-producing sIgG⁺ cells are lymphoblastoid cells (Horst et al., 2002; Lagresle et al., 1993). Thus, the effect of CNTF on Ig production was studied in purified sIgE⁺, sIgE[−], sIgG1⁺, sIgG1[−], sIgA1⁺ or sIgA1[−] B cells.

As shown in Table 3, while CNTF enhanced IgE production by sIgE⁺ B cells from atopic patients, CNTF failed to enhance IgG1 and IgA1 production by sIgG1⁺ B cells and sIgA1⁺ B cells, respectively. On the other hand, CNTF had no effect on sIgE[−] B cells, sIgG1[−] B cells or sIgA[−] B cells. Moreover, plasma cell marker CD38 was highly expressed on sIgE⁺ B cells, but

Table 2
Effect of CNTF on IgE production by sIgE[−] or sIgE⁺ B cells from atopic patients

Factor	IgE production (ng/ml)					
	AEDS		Allergic rhinitis		Bronchial asthma	
	sIgE [−] B	sIgE ⁺ B	sIgE [−] B	sIgE ⁺ B	sIgE [−] B	sIgE ⁺ B
Medium	<0.15	12.4 (2.2)	<0.15	5.2 (0.6)	<0.15	7.4 (0.9)
CNTF	<0.15	26.9 (3.0)*	<0.15	15.3 (1.8)*	<0.15	16.5 (1.8)*
IL-4 + α -CD40	6.8 (0.5)*	12.2 (2.1)	4.9 (0.4)*	5.4 (0.6)	4.1 (0.3)*	7.6 (1.0)
CNTF + IL-4	<0.15	27.2 (3.1)*	<0.15	15.5 (1.8)*	<0.15	16.9 (2.0)*
CNTF + α -CD40	<0.15	26.1 (2.9)*	<0.15	15.0 (1.6)*	<0.15	16.7 (1.9)*

Purified sIgE[−] B cells (sIgE[−] B) or sIgE⁺ B cells (sIgE⁺ B) from patients with AEDS (atopic eczema/dermatitis syndrome), allergic rhinitis, or bronchial asthma were cultured with indicated factors. CNTF was used at 1 ng/ml, IL-4 at 1000 U/ml, and anti-CD40 mAb (α -CD40) at 100 ng/ml.

Values are the means (SEM) from six experiments in each group of patient.

* Significant enhancement ($P < 0.01$) compared to cultures with medium.

Table 3
Effect of CNTF on Ig production by sIg[−] or sIg⁺ B cells from atopic patients

B cells	AEDS		Allergic rhinitis		Bronchial asthma	
	Medium	CNTF	Medium	CNTF	Medium	CNTF
<i>IgE production (ng/ml)</i>						
sIgE [−] B	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
sIgE ⁺ B	13.9 (2.6)	28.4 (2.7)*	6.3 (0.8)	18.0 (2.5)*	8.7 (1.1)	17.3 (2.4)*
<i>IgG1 production (ng/ml)</i>						
sIgG1 [−] B	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
sIgG1 ⁺ B	20.1 (3.2)	23.7 (3.4)	8.3 (1.9)	9.1 (2.0)	19.8 (3.8)	21.3 (3.9)
<i>IgA1 production (ng/ml)</i>						
sIgA1 [−] B	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
sIgA1 ⁺ B	15.1 (3.4)	13.5 (3.1)	7.5 (1.3)	7.9 (1.4)	11.5 (1.9)	10.6 (1.8)

Purified sIg[−] B cells (sIg[−] B) or sIg⁺ B cells (sIg⁺ B) from patients with AEDS (atopic eczema/dermatitis syndrome), allergic rhinitis or bronchial asthma were cultured with medium or CNTF (1 ng/ml), and production of IgE, IgG1 or IgA1 was measured.

Values are the means (SEM) from six experiments.

* Significant enhancement ($P < 0.01$) compared to cultures with medium.

not on other sIg⁺ B cells. The percentage (mean $\pm$ SEM) of CD38⁺ cells in sIgE⁺ B cells were $85 \pm 2\%$, while the percentage of CD38⁺ cells in sIgG1⁺ B cells and sIgA1⁺ B cells were $5 \pm 1\%$ and $3 \pm 1\%$, respectively, ($n = 5$).

3.5. Kinetics of the effect of CNTF on IgE production and proliferation in sIgE⁺ B cells

It is possible that enhancement of IgE production may be due to proliferation of sIgE⁺ B cells. Thus, the effect of CNTF on IgE production and proliferation in sIgE⁺ B cells from AEDS patients were studied. As shown in Figs. 2 and 3, CNTF enhanced IgE production on day 1 and 2 of culture without enhancement of proliferation. After 3 days of culture, both IgE production and proliferation were enhanced. These results indicated that CNTF had both enhancing effect of IgE production and proliferation. Similar findings were observed in sIgE⁺ B cells from patients with allergic rhinitis or bronchial asthma (data not shown).

The dose-response of CNTF on B cell proliferation was also studied. Purified sIgE⁺ B cells were cultured with medium or different concentrations of CNTF for 6 days. In four experiments, the mean % enhancement of OD value by CNTF were as follows: 5% by CNTF 0.01 ng/ml, 36% by CNTF 0.1 ng/ml, 96% by CNTF 1 ng/ml, and 85% by CNTF 10 ng/ml.

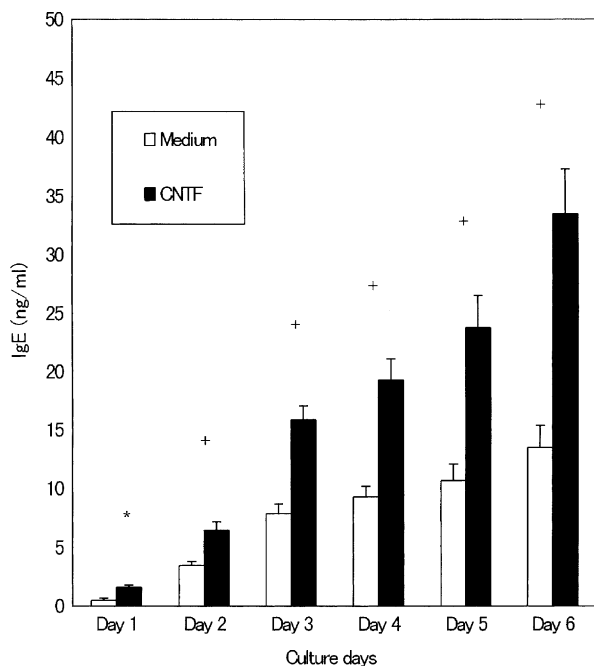


Fig. 2. Kinetics of the effect of CNTF on IgE production by sIgE⁺ B cells. Purified sIgE⁺ B cells from AEDS (atopic eczema/dermatitis syndrome) patients were cultured with medium or CNTF (1 ng/ml) for 1–6 days, and IgE production was measured. Values are the means $\pm$ SEM from six experiments. * Significant enhancement ($P < 0.05$) compared to cultures with medium. + Significant enhancement ($P < 0.01$) compared to cultures with medium.

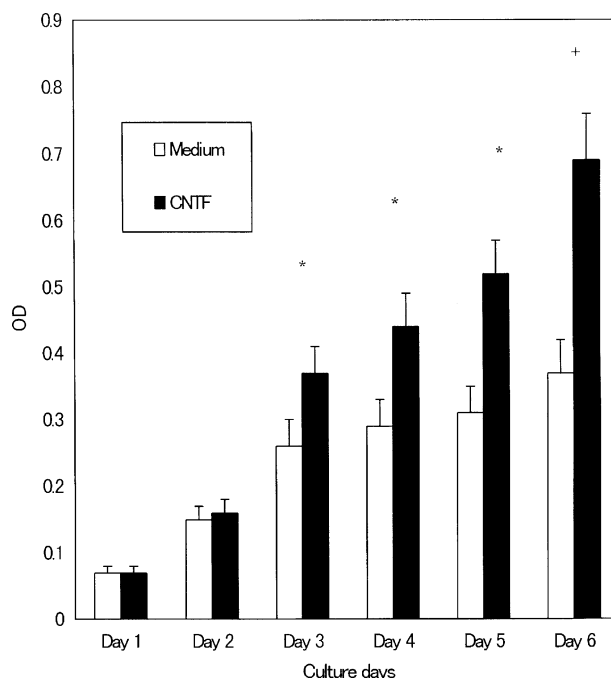


Fig. 3. Kinetics of the effect of CNTF on proliferation of sIgE⁺ B cells. Purified sIgE⁺ B cells from AEDS (atopic eczema/dermatitis syndrome) patients were cultured with medium or CNTF (1 ng/ml) for 1–6 days, and proliferation was measured by MTT assay. Values are the means $\pm$ SEM from six experiments. * Significant enhancement ($P < 0.05$) compared to cultures with medium. + Significant enhancement ($P < 0.01$) compared to cultures with medium.

4. Discussion

These results demonstrated that CNTF enhanced IgE production by B cells from atopic patients, while CNTF did not induce by B cells from non-atopic subjects. This was due to the fact that CNTF enhanced IgE production and proliferation in sIgE⁺ B cells from atopic patients, while CNTF had no effect on sIgE[−] B cells from non-atopic subjects. This indicates that CNTF acts on post-switched sIgE⁺ B cells and enhances IgE production as enhancing factor, but not as switching factor. This was specific to CNTF and was not mediated by IL-6, since CNTF effect was blocked by anti-CNTF mAb or anti-gp 130 mAb, but not by anti-IL-6 mAb.

In contrast to enhancement of IgE production, CNTF did not significantly enhance production of IgG1, IgG2, IgG3, IgG4, IgM, IgA1 or IgA2. This was directly demonstrated that CNTF did not enhance Ig production by sIgG1⁺ B cells or sIgA1⁺ B cells. Similarly, CNTF failed to enhance Ig production by sIgG2⁺, sIgG3⁺, sIgG4⁺, sIgM⁺, or sIgA2⁺ B cells (data not shown). This may be due to the fact that most of sIgE⁺ B cells are the plasma cells, while sIgG1⁺, sIgG2⁺, sIgG3⁺, sIgG4⁺, sIgM⁺, sIgA1⁺ or sIgA2⁺ B cells are lymphoblastoid cells in atopic patients (Horst et al., 2002; Kimata et al., 1996; Lagresle et al., 1993). Indeed, this was proved to be true, since percentage of CD38⁺

cells in sIgE+ B cells were around 85%, while those in sIgG1+ B cells or sIgA1+ B cells were less than 10%. Similarly, percentage of CD38+ cells in sIgG2+, sIgG3+, sIgG4+, sIgM+ or sIgA2+ B cells were less than 10% (our unpublished results). The fact that IgE-plasma cells had surface IgE was not surprising. It has been reported that up to 88% of cells in human IgE-plasmacytoma cell line have surface IgE (Sherr et al., 1989).

Kinetic experiments showed that CNTF had enhancing activity for both IgE-production and proliferation, and thus CNTF may be differentiation and proliferation factor for plasma cells. In accordance with this, CNTF induced proliferation of human myeloma cell line (Gu et al., 1996), and CNTF was measurable in 41.8% of patients with multiple myeloma (Wierzbowska et al., 1999). In addition, it has been reported that serum levels of CNTF were elevated in patients with systemic lupus erythematosus, in which immunoglobulin production was augmented (Robak et al., 1997). These results suggest that CNTF may also be involved in enhanced immunoglobulin production in vivo.

On the other hand, IgE-plasma cells in atopic patients are long-lived and produce IgE continually in vivo. Although the biological significance of CNTF in allergic diseases remains to be elucidated, it is tempting to speculate that CNTF may be involved in longevity of IgE-plasma cells. Moreover, CNTF was spontaneously produced in vitro by purified B cells from patients with AIDS (22.8 ± 3.5 pg/ml), allergic rhinitis (14.8 ± 1.4 pg/ml) or bronchial asthma (17.3 ± 2.4 pg/ml), while no CNTF (<8 pg/ml) was produced by B cells from non-atopic subjects ($n = 10$, manuscript in preparation). It is tempting to speculate that CNTF may be produced by B cells from atopic patients, which in turn stimulated sIgE+ B cells in atopic patients. Collectively, CNTF may be autocrine factor for sIgE+ B cells in allergic diseases. This possibility is currently under investigation.

References

- Boguniewicz, M., Jaffe, H.S., Izu, A., Sullivan, M.J., York, D., Geha, R.S., Leung, D.Y., 1990. Recombinant gamma interferon in treatment of patients with atopic dermatitis and elevated IgE levels. *Am. J. Med.* 88, 365–370.
- Gu, Z.-J., Zhang, X.-G., Hallet, M.-H., Lu, Z.-Y., Wijdenes, J., Rossi, J.-F., Klein, B., 1996. A ciliary neurotrophic factor-sensitive human myeloma cell line. *Exp. Hematol.* 24, 1195–1200.
- Horst, A., Hunzelmann, N., Arce, S., Herber, M., Manz, R.A., Radbruch, A., Nischt, R., Schmitz, J., Assenmacher, M., 2002. Detection and characterization of plasma cells in peripheral blood: correlation of sIgE+ plasma cells frequency with IgE serum titre. *Clin. Exp. Immunol.* 130, 370–378.
- Kimata, H., Yoshida, A., Fujimoto, M., Mikawa, H., 1993. Effect of vasoactive intestinal peptide, somatostatin, and substance P on spontaneous IgE and IgG4 production in atopic patients. *J. Immunol.* 150, 4630–4640.
- Kimata, H., 1995. High-dose intravenous γ -globulin treatment for hyperimmunoglobulinemia E syndrome. *J. Allergy Clin. Immunol.* 95, 771–774.
- Kimata, H., Lindley, I., Furusho, K., 1995. Effect of hydrocortisone on spontaneous IgE and IgG4 production in atopic patients. *J. Immunol.* 154, 3557–3566.
- Kimata, H., Fujimoto, M., Ishioka, C., Yoshida, A., 1996. Histamine selectively enhances human immunoglobulin E (IgE) and IgG4 production induced by anti-CD58 monoclonal antibody. *J. Exp. Med.* 184, 357–364.
- Kobayashi, H., Mizisin, A.P., 2000. CNTFR α alone or in combination with CNTF promotes macrophage chemotaxis in vitro. *Neuropeptides* 34, 338–347.
- Lagresle, C., Bella, C., Defrance, T., 1993. Phenotypic and functional heterogeneity of the IgD+ B cell compartment: identification of two major tonsillar B cell subsets. *Int. Immunol.* 5, 1259–1268.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. *J. Immunol. Methods* 65, 55–63.
- Robak, E., Sysa-Jedrzejowska, A., Stepień, H., Robak, T., 1997. Circulating interleukin-6 type cytokines in patients with systemic lupus erythematosus. *Eur. Cytokine Netw.* 8, 281–286.
- Sendtner, M., Carroll, P., Holtman, B., Hughes, R.A., Thoenen, H., 1994. Ciliary neurotrophic factor. *J. Neurobiol.* 25, 1436–1453.
- Shapiro, L., Panayotatos, N., Meydani, S.N., Wu, D., Dinarello, C.A., 1994. Ciliary neurotrophic factor combined with soluble receptor inhibits synthesis of proinflammatory cytokines and prostaglandin- E_2 in vitro. *Exp. Cell Res.* 215, 51–56.
- Sherr, E., Macy, E., Kimata, H., Gilly, M., Saxon, A., 1989. Binding the low affinity Fc ϵ R on B cells suppress ongoing human IgE synthesis. *J. Immunol.* 142, 481–489.
- Valerio, A., Ferraio, M., Dreano, M., Garotta, G., Spano, P., Pizzi, M., 2002. Soluble interleukin-6 (IL-6)receptor/IL-6 fusion protein enhances in vitro differentiation of purified rat oligodendroglial lineage cells. *Mol. Cell. Neurosci.* 21, 602–615.
- Wierzbowska, A., Urbanska-Rys, H., Robak, T., 1999. Circulating IL-6-type cytokines and sIL-6R in patients with multiple myeloma. *Br. J. Hematol.* 105, 412–419.