

Somatropin Promotes *Dermatophagoides farinae*-Specific IgE Generation Independently of IL-4 and IL-10

Takeshi Noma¹, Izumi Yoshizawa¹, Sumio Sugano², Norifumi Ogawa¹ and Yutaka Kawano¹

Interleukin (IL)-10 is an important mediator for inflammatory processes and the immune system.^{1,2} While IL-10 was originally shown to suppress the production of IFN- γ and IL-2 by Th1 cells,^{1,3} it has also been found to inhibit proliferation and cytokine responses in T cells.⁴ IL-10 blocks the binding of CD28 to B7.1 receptors,⁴ thus inhibiting IgE synthesis.⁵ IL-10 also decreases IL-4-induced IgE switching.⁶

In contrast, when peripheral mononuclear cells were activated with anti-CD40 and IL-4, IL-10 was found to enhance IgE production by increasing the synthesis of IL-6.⁷ Addition of IL-10 to purified human tonsillar B cells stimulated with anti-CD40 and IL-4 enhanced B cell proliferation and IgG production, but not IgE production.⁷ In a murine model spleen cells, primed with antigen, also produced a significant level of antigen-specific IgE on restimulation with the same

SUMMARY Interleukin (IL)-10 accelerates the IgE production of anti-CD40- and IL-4-stimulated PBMC by enhancing the IL-6 production of T lymphocytes or antigen-primed spleen cells, in addition to its role as a regulator of the inflammatory responses. To further investigate the mechanisms enhancing IgE synthesis, we determined the effect of somatropin as well as IL-10 on the secretion of *Dermatophagoides farinae* (Df)-specific IgE by K7 cells, which originate from an EBV-immortalized cell line. Df-pulsed autologous T cells, as well as the supernatants of these cultures, increased the synthesis of Df-specific IgE. Antigen-specific IgE was also enhanced when K7 cells were treated with anti-CD40 antibody and with both IL-4 and IL-10, or with IL-4 and IL-10 without anti-CD40 antibody. The treatment of K7 cells with anti-CD40 antibody and IL-4, or anti-CD40 antibody and IL-10 did not increase IgE production. The Df-specific IgE activity of the supernatants of K7 cells treated with somatropin alone was increased significantly although somatropin did not show any additive effect on the IgE production of anti-CD40 antibody-treated cells. The results indicate that IL-10, a Th2-type cytokine, directly affects the mature B cells that produce IgE, and that the secretion of IgE is increased by treatment with IL-10 in cells that are stimulated with anti-CD40 and IL-4 at the level of the EBV-immortalized cell line, which has already switched to IgE production. Somatropin similarly stimulates activated mature B cells to enhance their production of antigen-specific IgE without class switching, independently of IL-4 and IL-10.

antigen with IL-10 *in vitro*.⁸ However, IL-10 did not stimulate the spontaneous secretion of IgE from antigen-primed B cells.⁸

Somatropin (GH, growth hormone) was reported to induce the

production of IgE and IgG4 by purified surface IgE⁺, surface IgG4⁺ B

From the ¹Departments of Pediatrics, Kitasato University School of Medicine, Kanagawa, ²Department of Virology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Correspondence: Takeshi Noma

cells by means of class-switching in the presence of the anti-CD40 antibody.⁹

In order to investigate the mechanisms that enhance the IgE synthesis by mature B cells, we determined the effect of somatropin and IL-10 on the secretion of Df-specific IgE by EBV immortalized cell lines, which had already switched to IgE production.

SUBJECTS AND METHODS

Patient

PBMC were obtained from a 12-year-old Japanese patient with active bronchial asthma. The diagnostic criteria for bronchial asthma used were those defined by the American Thoracic Society. This patient had recurrent episodes of asthmatic attacks, as well as positive skin reactions to house dust mite and Df antigen (mite 1, Torii & Co, Ltd. Tokyo, Japan) as defined by an immediate wheal response to a skin prick test (positive response

10 mm mean diameter). The patient was suffering from more than 10 mild or moderate attacks per year, despite medication with theophylline (15-20 mg/kg/day), a β_2 -agonist or both. Disease severity and severity of acute exacerbations were estimated according to the Guidelines for the Diagnosis and Management of Asthma. This patient's total serum IgE and IgE score against mite antigen,¹⁰ as determined by RAST, were 790 IU/ml and 5, respectively. At the time of examination the patient was not experiencing an asthmatic attack or taking any oral corticosteroids or anti-allergic agents; e.g. ketotifen.¹¹ Informed consent was obtained from

the patient and from the parents. All investigations were formally approved by our Institutional Review Board.

Preparation of antigen-binding B cells

One million peripheral blood mononuclear cells (PBMC), isolated from heparinized peripheral blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation,¹² were added to 10 μ g/ml Df antigen and cultured for 2 days (37°C, 10% CO₂) in 1 ml RPMI-1640 medium containing 40 μ g/ml gentamycin and 10% heat-inactivated autologous serum. To collect non-T cells, the suspensions were mixed with sheep erythrocytes (SRBC) at a ratio of approximately 1:100 in FCS, centrifuged at 200 x g for 5 minutes to obtain tight cell contact, and kept in ice water for 1 hour. The cells were gently resuspended and centrifuged over a Ficoll-Hypaque density gradient; rosette-forming cells were separated by sedimentation and non-T cells were harvested from the interface.

To enrich the population of Df-binding non-T cells, the cells were allowed to adhere to tissue culture dishes (Corning, tissue culture dish 25020, 100 x 20 mm) for 16 hours in 4 ml RPMI-1640 medium containing 40 μ g/ml gentamycin and 10% heat-inactivated autologous serum. Weakly adherent and non-adherent cells were collected by swirling the plate. Four milliliters of this suspension, containing approximately 1×10^6 cells, were added to a Df-coated tissue culture dish (Corning), prepared by incubating the dish for 24 hours at 4°C with 100 μ g/ml Df antigen and

washing 5 times with RPMI-1640 medium. The plates were swirled, the non-adherent cells were removed after 1 hour incubation at room temperature, and the adherent cells were cultured for 16 hours in RPMI-1640 containing 10% heat-inactivated autologous serum and collected by flushing the plate with a Pasteur pipet. The cells were then cultured for an additional 16 hours (37°C, 10% CO₂) in 1 ml RPMI-1640 containing 40 μ g/ml gentamycin and 10% heat-inactivated autologous serum.

Immortalization

Df-binding B cells (1×10^5) were infected for 1 hour at 37°C with 0.5 ml of culture supernatant of the EBV-producing B95.8 marmoset cell line.¹³ The cultures were diluted 10 fold with RPMI-1640 supplemented with 10% FCS (GIBCO) and 50 μ M of 2-mercaptoethanol and cultured for an additional 16 hours. The cells were cultured for several days further until cell growth was confirmed.

Production of EBV-transformed B cell lines producing Df-specific immunoglobulin

EBV-infected B cells were cultured for 3 days in tissue culture dishes (Corning), and single cells were collected under the microscope with fine Pasteur pipets. Each colony was cultured until cell growth was confirmed and culture supernatant was collected for detecting Df-specific immunoglobulin.

Measurement of Df-specific IgM, IgE and IgG

The wells of flat-bottomed,

96-well microplates (Limbro, McLean, VA) were coated with Df antigen; 50 µg/ml in 50 µl of carbonate buffer, pH 9.6. After an overnight coating with antibody at 4°C in a humidified atmosphere, the plates were washed with phosphate-buffered saline (PBS), and the uncoated surfaces of each well were saturated for 1 hour at 37°C with 300 µl of 0.25% gelatin (Wako, Osaka, Japan). After another wash, a 50 µl sample was placed in each well, and the plates were incubated for 16 hours at 4°C. After extensive washing of the plates, the samples were allowed to react with 50 µl murine anti-human IgE (Yamasa, Choshi, Japan), anti-human IgM (Tago, Inc., Burlingame, CA), or anti-human IgG (Tago) monoclonal antibody, and then with 50 µl biotinylated goat anti-mouse IgG antibody (Tago). The samples were reacted with 50 µl of a 1,000-fold dilution of streptavidin-horseradish peroxidase conjugate (BRL, Gaithersburg, MD) and *o*-phenylenediamine (BRL, Gaithersburg, MD), and the optical density (OD) at 492 nm was read with an enzyme-linked immunosorbent assay (ELISA) reader (APR-A4, Tosoh, Tokyo, Japan). Prior to its use as a second antibody, the goat anti-mouse IgG antibody was passed through a human Ig-coupled Sepharose 4B column to prevent nonspecific binding with human Ig, after which it was diluted 1:200. Human sera with high titers of Df-specific IgE, IgM and IgG, as well as purified human IgE (derived from human myeloma cells), IgM and IgG, were utilized as standards. All results were expressed as pg/ml of Ig. The sensitivity of this assay was 40 pg/ml. The specific anti-Df IgE levels in human serum were determined by

RAST.¹⁰

Proliferation of transformed B cell lines

Using 96-well microplates (NUNC, Roskilde, Denmark), 2 x 10⁴ cells were cultured in the presence of Df in RPMI-1640 medium plus 40 µg/ml gentamycin and 10% heat-inactivated pooled human serum. After 24 or 48 hours of culture (37°C, 10% CO₂), 0.5 µCi ³H-TdR was added to each well and the cells were incubated for an additional 6 or 18 hours. The cells were harvested, and ³H-TdR uptake was quantified by liquid scintillation counting.

Flow cytometry

Surface immunoglobulin isotypes on the EBV-infected B cells were evaluated by a two-color staining technique. Briefly, cells were reacted with FITC-labeled mouse anti-human IgM (G20-127, Pharmingen, San Diego, CA) or anti-human IgG monoclonal antibody (HP6017, Pharmingen) at 4°C for 30 minutes and then washed twice with PBS containing 2% FCS and 0.1% NaN₃. The cells were incubated for 30 minutes at 4°C with biotinylated mouse anti-human IgE monoclonal antibody (G7-26, Pharmingen), again washed with PBS containing 2% FCS and 0.1% NaN₃, and resuspended in PBS containing a streptavidin-phycoerythrin conjugate (Pharmingen). After a 20 minute incubation at 4°C, the cells were washed with PBS. As negative controls, we used FITC-labeled and biotin-conjugated mouse IgG1, kappa, and monoclonal immunoglobulin isotype standard (Pharmingen), respectively. Cells were analyzed on a FACScan flow

cytometer (Becton Dickinson, San Jose, CA). True typical forward and side scatter gates for lymphocytes were set to exclude dead cells; 10,000 events within this gate were acquired per sample. Two-parameter histograms demonstrating surface Igs staining were created using CellQuest software (Becton Dickinson), and quadrant statistics were calculated on the basis of the staining of specifically blocked negative controls.

To rule out the possibility that the immunoglobulins on the B-cell surface were in part cytophilic, we used specifically blocked negative controls with isotype-matched mouse IgG1. Cells that stained with anti-IgE antibody were stained with anti-IgM or anti-IgG antibody in an inverse order. The results by both methods were similar (IgM⁺E⁺ cells, 47.5% and 49.1%; see Result).

Construction of a cDNA library

Double stranded cDNAs were synthesized from 1 µg of polyA⁺ RNA extracted from K7 cells using an Amersham cDNA Synthesis Kit (Amersham) and oligo-dT primer. The cDNAs were blunt-ended with T4 DNA polymerase, and a *Bst* XI adapter (Invitrogen) was ligated to each end. After ligation, cDNAs longer than 800 bp were isolated and ligated into the *Bst* XI site of vector. The cDNA library was screened without further amplification.

PCR

Following 1st strand synthesis, the RNA was degraded by incubation in 0.015 N NaOH at 65°C for 1 hour. A 5 µl aliquot of

the reverse transcription product was added to 10 μ l of PCR reaction mixture containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, 0.5 U *Taq* polymerase (Perkin Elmer, Foster City, CA), 10 pM of antisense primer (5'-CGG AAT TCA AGC ACT GGC TGT CAG ACC GCA CCT-3'), and 10 pM of sense primer (5'-CGT CTA GAA GGC ATG AAG TTC TGG ATC AGG CA-3'). For the 5'-AGC ACT GGC TGT CAG ACC GCA CCT-3' portion of the antisense primer we used positions 850 to 874 in the nucleotide sequence of the C ϵ gene segment cDNA from a λ - ϵ -1.2 clone obtained from the human myeloma cell line 266B1 cDNA to mRNA.¹⁴ The 5'-CGG AAT TCA-3' portion of the antisense primer included a site representing a suitable restriction enzyme cut point. For the 5'-A GGC ATG AAG TTC TGG ATC AGG CA-3' portion of the sense primer we used positions 1,504 to 1,528 in the nucleotide sequence of the same C ϵ gene segment. The 5'-CGT CTA GA-3' portion of the sense primer also included a suitable restriction enzyme cut point. Amplification was performed in a Perkin Elmer 9600R thermal cycler. Following an initial denaturation at 94°C for 5 minutes, the amplification protocol consisted of 40 cycles of denaturation (1 minute at 94°C), annealing (1 minute at 55°C), and extension (2 minutes at 72°C), followed by a final extension at 72°C for 5 minutes. Amplification of the IgE constant region resulted in the generation of a 510 bp product from K7 cells and a 680 bp product from placental DNA as genomic controls. The 510 bp product was verified by DNA sequencing.

Effect of T cells on antigen-specific IgE production

EBV-transformed B cells were continuously cultured in medium reconstituted with irradiated autologous T cells or T cell supernatants. After 3 days, the supernatants were harvested for IgE analysis.

Autologous T cells had been isolated on polystyrene resin columns¹⁵ following 16 hours activation with a 0.01 or 1 μ g/ml dose of Df antigen and irradiation (15 Gy). For the collection of the culture supernatants, 10⁶ Df (1 μ g/ml)-pulsed T cells were suspended in 1 ml RPMI-1640 medium containing 40 μ g/ml gentamycin and 10% heat-inactivated pooled human serum in

a 5 ml culture tube (Falcon) and then cultured for 3 days (37°C, 10% CO₂).

Effect of cytokines on production of Df-specific IgE

The EBV-transformed B cells were treated with anti-CD40 antibody (Serotec, Oxford, UK) and/or a cytokine. The latter included G-CSF (Kyowa Hakko Kogyo Co. Ltd. Tokyo, Japan), IL-4 (Genzyme, Cambridge, MA, USA), IL-10 (kindly provided by Dr H. Ishida and M. Howards, the DNAX Research Institute, Palo Alto, CA, USA), IL-13 (Pepro Tech EC Ltd., London, England), growth hormone (somatropin, Eli Lilly and Company, Indianapolis, Indiana, USA) and IFN- γ (Takeda Co. Ltd. Tokyo,

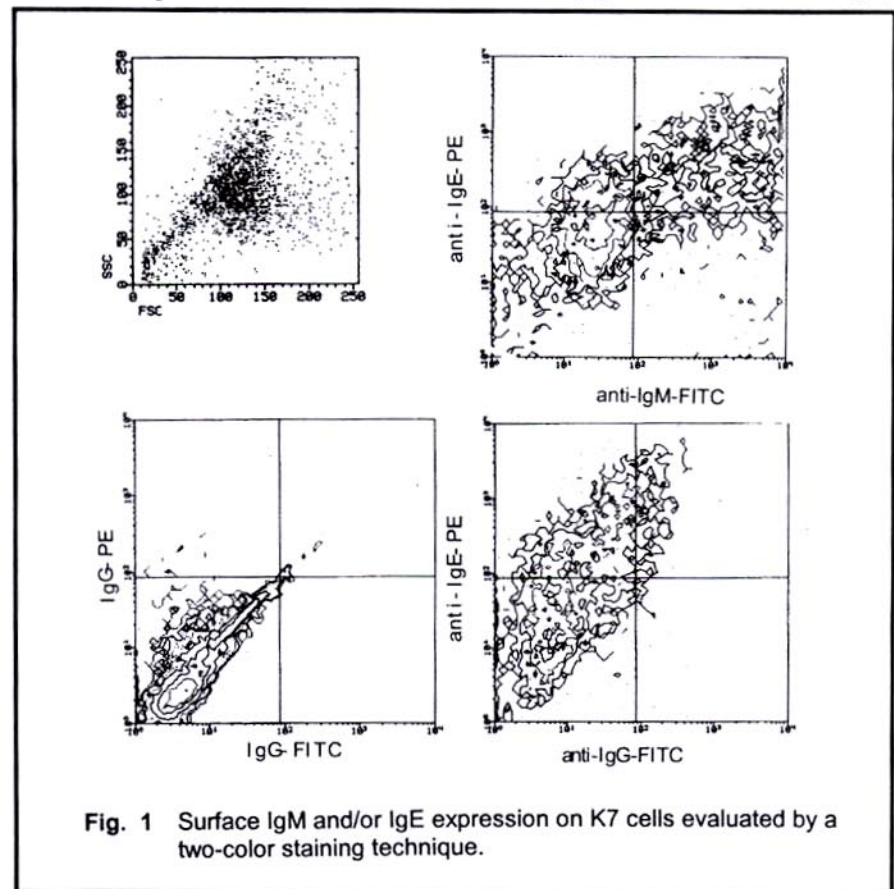


Fig. 1 Surface IgM and/or IgE expression on K7 cells evaluated by a two-color staining technique.

Japan). Cells were cultured for 3 days at 3×10^5 cells/well in 24-well flat-bottom plates (Becton Dickinson, San Jose, CA) in a final volume of 500 μ l. The culture supernatant was collected for an assay of specific IgE activity.

Data analysis

The data obtained were analyzed with the two-tailed Student's t-test for comparison with the control data. Any *p* value < 0.05 was considered significant.

RESULTS

Production of Df-specific IgM

Macroscopic evidence of the growth of our EBV-transformed cells was observed after 1-2 weeks of culture. To obtain B cell clones secreting Df-specific Ig, the transformed cells were cultured for several additional days, and single cells were picked and cultured in 96-well microplates. Growth of these colonies was observed after 1-2 weeks in approximately 90% of the wells. When we tested the culture supernatant of 22 clones (of the 26 maintained) for the ability to produce Df-specific Igs, we found that 12 of these clones secreted significant amounts of Df-specific IgM. To further evaluate these cells, we selected two lines secreting Df-specific IgM (K7 and K40), as well as one (k42) that did not secrete IgM (data not shown). Secretion of small amounts (approximately 0.5-1 ng/200 μ l / 10^5 cells) of IgE, but not IgG, was detected after further culture of the K7 cells.

Following stimulation of K40 cells with Df antigen, the amount of

antigen-specific IgM produced decreased compared to unstimulated cells. The amount of Df-specific IgM produced by K7 cells showed a decrease upon further culture (data not shown). In contrast, stimulation with Df antigen increased the production of Df-specific IgM by K42 cells (data not shown).

To evaluate the ability of autologous T cells to affect the production of IgM by the K7, K40 and K42 lines, 1×10^5 cells from each line were cultured with $1 \times$ or $3 \times$ Df (10 μ g/ml)-pulsed autologous T cells (15 Gy irradiation). At various

times culture supernatants were collected and the amount of Df-specific IgM was measured. We found that 10^5 autologous T cells enhanced the production of Df-specific IgM by the same number of K7 and K40, but not of K42, cells (data not shown). On the bases of these results, we selected the K7 line for further evaluation of Df-specific immune responses.

Proliferation of K7 cells

To evaluate the proliferation of K7 cells, these cells were cultured for 24 or 48 hours with serial

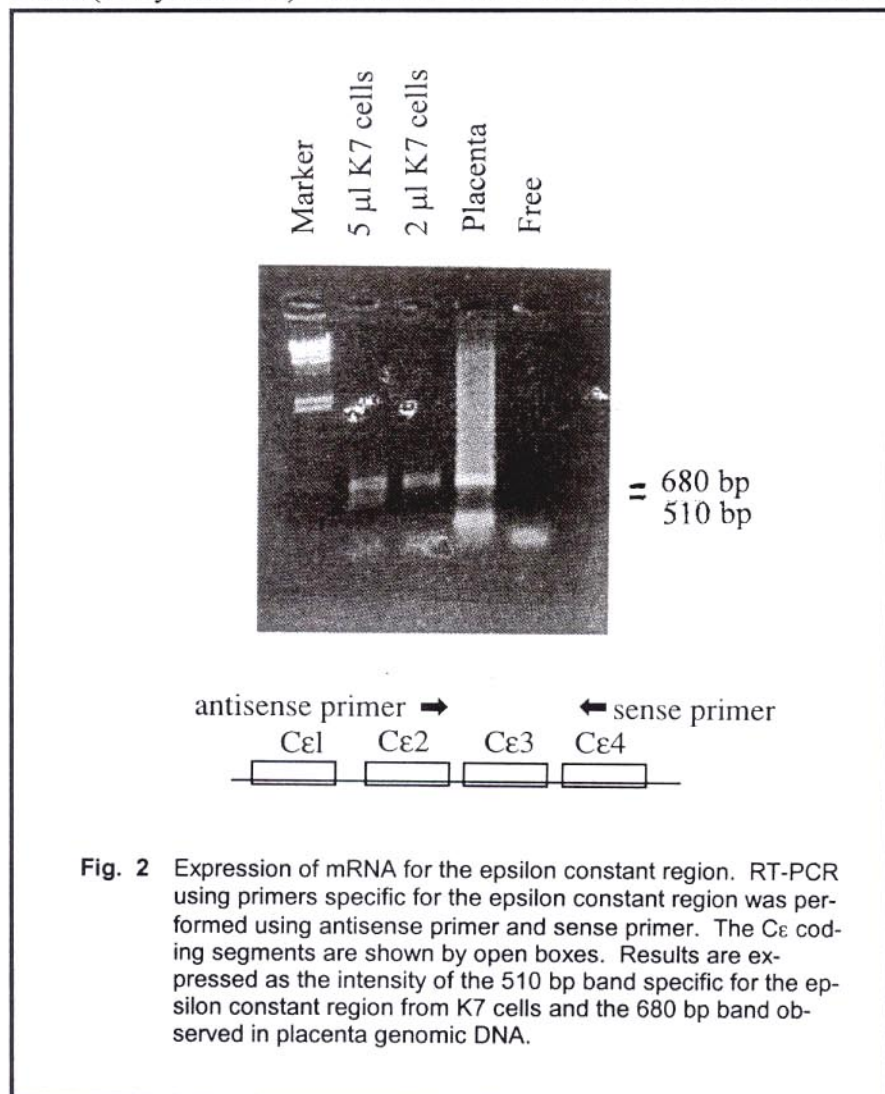


Fig. 2 Expression of mRNA for the epsilon constant region. RT-PCR using primers specific for the epsilon constant region was performed using antisense primer and sense primer. The C ϵ coding segments are shown by open boxes. Results are expressed as the intensity of the 510 bp band specific for the epsilon constant region from K7 cells and the 680 bp band observed in placenta genomic DNA.

dilutions of Df antigen. The ^3H -TdR uptake by the cells was measured for another 6 or 18 hours. While no increased proliferation of the K7 cells was observed with 0.01 or 0.1 $\mu\text{g/ml}$ Df antigen, significant proliferation was detected when the K7 cells were incubated with 1, 10 or 50 $\mu\text{g/ml}$ Df antigen for 48 hours (data not shown).

Production of Df-specific IgE

K7 cells were pulsed with graded doses of Df antigen for 4 hours. After 3 days, Df-specific IgE was measured in the supernatant. We detected 7.3 ± 1.4 (SE) ng/ml Df-specific IgE per 5×10^5 cells ($n = 6$) which were not exposed to Df antigen (data not shown). The amount of antigen-specific IgE was augmented in cells stimulated with Df antigen (data not shown), but this increase was not statistically significant. The amount of Df-specific IgE was reduced when the supernatant was absorbed with anti-IgE monoclonal antibody or Df antigen (data not shown). These results confirmed that the supernatants of K7 cells cultured for 3 days contained Df-specific IgE.

Analysis of cell surface markers

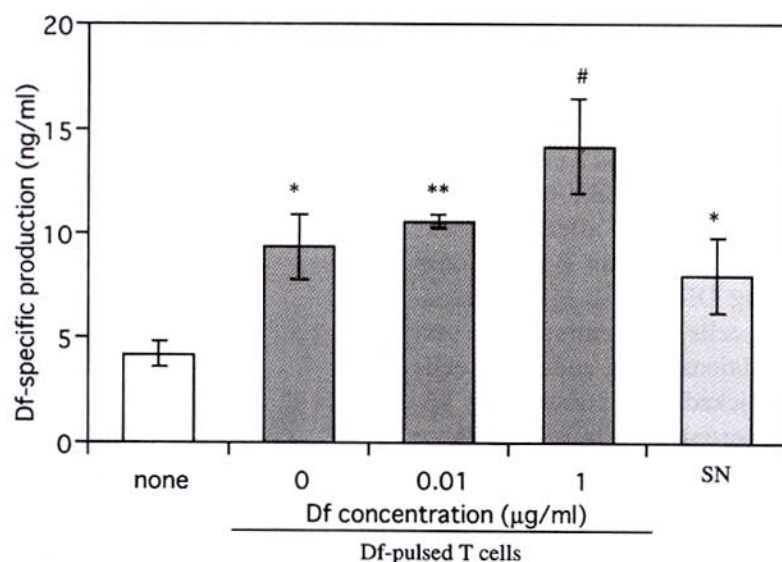
FACS analysis showed that fluorescence intensity of surface IgM^+ , IgE^+ , IgD^- , IgA^- and IgG^- cell populations was higher than that of specifically blocked negative controls. In addition, the fluorescence intensity of IgM^+ cell populations stained with biotin-conjugated anti-IgE antibody and then with avidin-conjugated rhodamine was higher than that of cells stained with isotype control antibody (IgM^+E^+ cells: 47.5%) (Fig. 1), and the fluo-

rescence intensity of IgE^+ cell populations stained with FITC-conjugated anti-IgM antibody was higher than that of cells stained with isotype control antibody (IgM^+E^+ cells: 49.1%). These results suggest that IgM^+E^+ K7 cells secrete IgM and IgE.

Expression of mRNA encoding the epsilon constant region

To determine whether the expression of IgE-specific mRNA would be detected in K7 cells fol-

lowing stimulation with Df antigen, we performed RT-PCR using primers specific for the epsilon constant region. The intensity of the 510 bp band specific for that region was observed. The band intensity was different from that of the 680 bp band observed in placental genomic DNA (Fig. 2). The cDNA of a 510 bp product was sequenced to verify its identity (data not shown). These results indicated that the Df-antigen-stimulated K7 cells expressed IgE-specific mRNA.



□ Df-specific IgE secreting cells
 ■ added to Df-pulsed autologous T cells
 ■ added to supernatants from Df-pulsed allogeneic patient T cells (SN)

* : $p < 0.01$, ** : $p < 0.001$ (as compared to IgE secreting cells alone)

: $p < 0.05$ (as compared to unstimulation or 0.01 $\mu\text{g/ml}$ Df stimulation)

Data shown are means \pm standard errors of 4 to 8 independent experiments.

Fig. 3 Df-pulsed T cells facilitate the secretion of Df-specific IgE by activated B cells. EBV-transformed B cells, K7 were continuously cultured with autologous T cells or T cell supernatants and the supernatants were harvested for IgE analysis. Autologous T cells had been isolated on polystyrene resin columns following 16 hours activation with a 0.01 or 1 $\mu\text{g/ml}$ dose of Df antigen. For collection of the culture supernatants, 10^6 Df (1 $\mu\text{g/ml}$)-pulsed T cells (15 Gy irradiation) were cultured for 3 days.

Df-pulsed T cells facilitate the secretion of Df-specific IgE by activated B cells

When we cultured K7 cells with Df-pulsed autologous T cells for 3 days, we found that the autologous T cells enhanced the secretion of Df-specific IgE (Fig. 3). This effect was dose-responsive, being higher in T cells pulsed with 1 $\mu\text{g}/\text{ml}$ Df than in cells pulsed with 0.01 $\mu\text{g}/\text{ml}$ Df (Fig. 3). In addition, when we cultured K7 cells with the culture supernatants of Df-pulsed autologous T cells, we observed a similar augmentation of Df-specific IgE secretion (Fig. 3).

Anti-CD40 and/or cytokine affect Df-specific IgE secretion

K7 cells were treated with saturating amounts of anti-CD40, or were added to a cytokine such as IL-4, IL-13, GH (somatropin), IL-10, G-CSF or IFN- γ , and cultured for 3 days. Df-specific IgE activity in the supernatants of the K7 cells treated with somatropin showed a significant increase at doses of 35 and 350 ng/ml (Fig 4). The following stimulators did not increase the Df-specific IgE activity: anti-CD40 antibody, IL-4 (10, 50, 100, and 250 U/ml), IL-13 (10 ng/ml), IL-10 (100 U/ml), G-CSF (180 ng/ml) or IFN- γ (100 U/ml).

Treatment of K7 cells with anti-CD40 antibody, followed by culture with both IL-4 (100 U/ml) and IL-10 (100 U/ml), extremely enhanced the production of Df antigen-specific IgE (Fig. 5A). This effect was not observed when the cells were treated with either of these

2 cytokines alone. When the cells were treated with IL-4 and IL-10, but not with anti-CD40 antibody, however, the production of Df-specific IgE was increased (Fig. 5A). When the anti-CD40-treated K7 cells were added to somatropin instead of IL-4 plus IL-10, IgE activity was similarly increased although no additive effect was observed (Fig. 5B). The results indicate that somatropin as well as IL-4 plus IL-10 enhanced the production of IgE by activated B cells that spontaneously secreted antigen-specific IgE, whereas somatropin does not assist the production of IgE by the cells activated through CD40 molecules.

DISCUSSION

We observed that the culture supernatants of autologous, Df-pulsed T cells, as well as the cells themselves, augmented the production of antigen-specific IgE by K7 cells. The activity of the culture supernatants indicated that a secreted protein, e.g. a cytokine, may be responsible. We therefore assayed the effect of the cytokines as well as that of anti-CD40 antibody, on Df-specific IgE production. We investigated enhanced production of such Df-specific IgE in cells treated with anti-CD40 antibody and with both IL-4 and IL-10. The CD40 ligand has been shown to deliver a

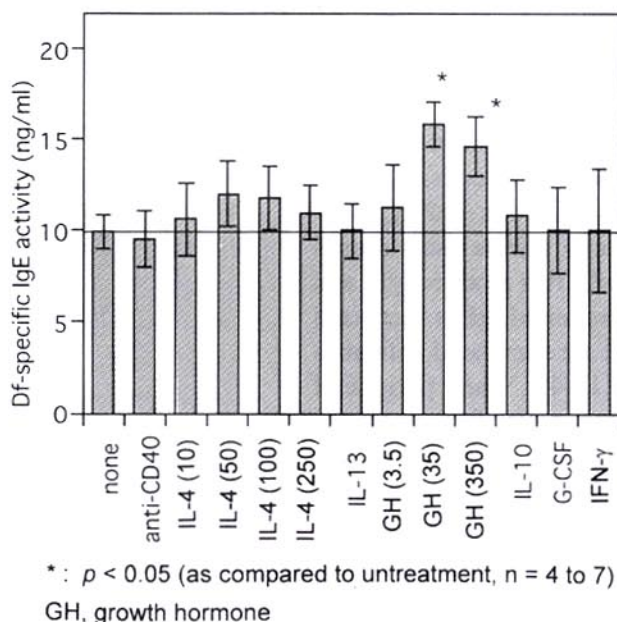


Fig. 4 Somatropin enhances the secretion of Df-specific IgE. K7 cells were added to serial dilutions of GH (somatropin), IL-4, IL-13, IL-10, G-CSF and IFN- γ , and cultured for 3 days. Culture supernatant was collected for the analysis of specific IgE activity. IL-4 (10), IL-4 (50), IL-4 (100), IL-4 (250): added to 10, 50, 100 and 250 U/ml; IL-13: added to 10 ng/ml; GH (somatropin) (3.5), GH (35), GH (350): added to 3.5, 35 and 350 ng/ml; IL-10: added to 100 U/ml; G-CSF: added to 180 ng/ml; IFN- γ : add to 100 U/ml.

contact-dependent signal to B cells, which, in the presence of IL-4 drives isotype switching to IgE.¹⁶ Since the treatment of K7 cells with IL-4 and anti-CD40 antibody did not increase IgE production, however, these agents probably did not drive isotype switching to IgE. Moreover, IL-10 decreases epsilon transcript expression and IgE production induced by IL-4 when added during the first 3 days of *in vitro* culture,⁶ suggesting that IL-10 did not affect IL-4-induced IgE switching in K7 cells.

Thus our B cell line has likely switched to the production of IgE. We have shown here that the secretion of IgE by mature B cells that spontaneously secrete IgE was enhanced by treatment with IL-4 and IL-10, but not by IL-10 alone. Since IL-10 also plays a role in stimulating the proliferation and differentiation of B cells,¹ our results suggest that this Th2 type cytokine directly affects the cultured mature B cells that produce IgE, and that the secretion of IgE is increased in cells that are stimulated with anti-CD40 and IL-4. When peripheral mononuclear cells from patients with mite-sensitive asthma were stimulated with anti-CD40 antibody and IL-4, the level of IL-10 synthesis was increased.⁷ IL-10 may block the *de novo* synthesis of IgE,^{4,5} whereas it may trigger positive feedback mechanisms in the IgE production as shown in the study.

Since somatropin induces the production of IgE and IgG4 by purified surface IgE⁺, IgG4⁺ B cells by means of class-switching in the presence of the anti-CD40 antibody according to an IL-4- and IL-13-independent mechanism,⁹ the effects

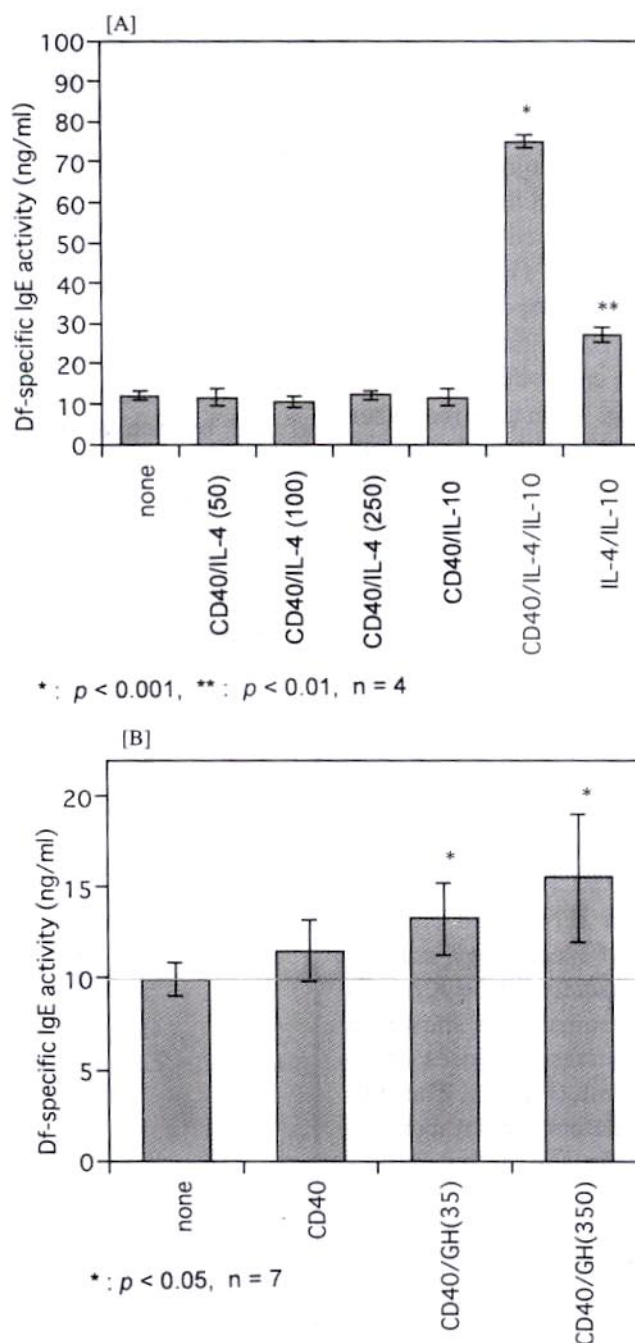


Fig. 5 Anti-CD40 antibody and cytokines enhance the secretion of Df-specific IgE. EBV-transformed B cells were treated with anti-CD40 antibody and cytokines, including IL-4 and IL-10 [A] and GH [B], and cultured for 3 days. Culture supernatant was collected for the analysis of specific IgE activity. IL-4 (50), IL-4 (100), IL-4 (250): added to 50, 100 and 250 U/ml, respectively; IL-4: add to 100 U/ml; IL-10: add to 100 U/ml; GH (somatropin) (35), GH (350): added to 35 and 350 ng/ml.

of somatropin on IgE generation were investigated. Purified surface IgE⁺, but not surface IgE⁻ B cells from atopic patients spontaneously produced IgE.⁹ Somatropin with anti-CD40 monoclonal antibody failed to increase IgE production of the surface IgE⁺ B cells.⁹ However, in the present study, the Df-specific IgE activity of the supernatants of K7 cells treated with somatropin alone was increased significantly although somatropin did not show an additive effect on the IgE production of the anti-CD40 treated cells. The present results suggest that somatropin causes the activated mature B cells to enhance their production of antigen-specific IgE without class switching.

In a previous study, IFN- γ , which counteracts the IL-4- and IL-13-induced production of IgE and IgG4, had no effect on induction by somatropin.⁹ In contrast to that report, we found that somatropin induced the production of IgE by mature B cells, being regulated by IFN- γ , as well as by a low concentration of IL-12 (unpublished data, 2001).

For studies of the synthesis of antigen-specific IgE, as well as an analysis of the mechanisms underlying its regulation, which have been hampered by the small amount of IgE produced *in vitro* by peripheral mononuclear cells from allergic patients, we established B cell lines secreting *Dermatophagoides farinae*-(Df) specific IgE from peripheral mononuclear cells extracted from a patient with mite-sensitive asthma by immortalization with the Epstein-Barr virus (EBV) according to the previous report.^{13,17,18}

To select cells secreting Df-specific Ig at high cloning efficiency, we enriched the population of Df-binding B cells and transformed the latter with EBV. At the first stage, we screened clones for cells spontaneously producing Df-specific IgM since EBV-transformed cells produce IgM, and then selected cells secreting Df-specific IgE by assaying for reduced dependence on antigenic stimulation. The Df-specific IgM production by these cells was maintained by the addition to the culture of autologous T cells pulsed with Df. We identified two lines, K7 and K40 that spontaneously secreted Df-specific IgE. Although K7 cells proliferated in response to Df antigen, the production of Df-specific IgE was not augmented. RT-PCR confirmed that K7 cells express message encoding IgE, while FACS verified the expression of surface IgM and IgE, but not IgG or IgA, on K7 cells.

In this study we investigated the effect of IL-10 as well as somatropin on the production of antigen-specific IgE by activated mature B cell lines. When these *in vitro* observations are applied to the clinical setting, one may hypothesize that a patient with prolonged asthmatic attacks will produce large amounts of IL-4 and IL-10 in the bronchial mucosa by activation of such immune cells as Th2 cells, mast cells, eosinophils and monocytes/macrophages induce an increased production of IgE by mature B cells during the allergic inflammation, and thus boost the IgE generation in an IL-4- and IL-13-independent manner.

ACKNOWLEDGMENT

This research was supported in part by a grant from the Ministry of Education, Culture and Science, Japan.

REFERENCES

1. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. *Annu Rev Immunol* 1993; 11: 165-90.
2. Akdis CA, Blaser K. IL-10-induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy. *FASEB J* 1999; 13: 603-9.
3. Lalani I, Bhol K, Ahmed AR. Interleukin-10: biology, role in inflammation and autoimmunity. *Ann Allergy Asthma Immunol* 1997; 79: 469-83.
4. Schandene L, Alonso-Vega C, Willems F, *et al.* B7/CD28-dependent IL-5 production by human resting T cells is inhibited by IL-10. *J Immunol* 1994; 152: 4368-74.
5. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin-10 in specific immunotherapy. *J Clin Invest* 1998; 102: 98-106.
6. Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. *J Immunol* 1998; 160: 3555-61.
7. Uejima Y, Takahashi K, Komoriya K, Kurozumi S, Ochs HD. Effect of interleukin-10 on anti-CD40- and interleukin-4-induced immunoglobulin E production by human lymphocytes. *Int Arch Allergy Imm* 1996; 110: 225-32.
8. Ohmori H, Kanda T, Takai T, Hikida M. Induction of antigen-specific IgE response in murine lymphocytes by IL-10. *Immunol Lett* 1995; 47: 127-32.
9. Kimata H, Fujimoto M. Growth hormone and insulin-like growth factor I induce immunoglobulin (Ig)E and IgG4 production by human B cells. *J Exp Med* 1994; 180: 727-32.
10. Miyamoto T, Johansson S, Ito K, Horiuchi Y. Atopic allergy in Japanese subjects: studies primarily with radioallergosorbent test. *J Allergy Clin Immunol* 1974; 53: 9-14.

11. Noma T, Yoshizawa I, Baba M, Yata J, Akasaka T, Maeda K. Effect of ketotifen on antigen-induced interleukin 2 (IL-2) responsiveness in lymphocytes from patients with atopic dermatitis and/or bronchial asthma. *Int J Immunopharmacol* 1990; 12: 269-77.
12. Boyum A. Separation of lymphocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968; 21: 77-90.
13. Bende RE, Jochems GJ, Frame TH, *et al.* Effects of IL-4, IL-5, and IL-6 on growth and immunoglobulin production of Epstein-Barr virus-infected human B cells. *Cell Immunol* 1992; 143: 310-23.
14. Flanagan JG, Rabbitts TH. The sequence of a human immunoglobulin epsilon heavy chain constant region gene, and evidence for three non-allelic genes. *EMBO J* 1982; 1: 655-60.
15. Noma T, Kawano Y, Yoshimura N, Abe T, Inagaki K, Yata J. A rapid isolation technique of unmodified human T cells on a polystyrene resin column. *Eur J Immunol* 1985; 15: 833-7.
16. Fuleihan R, Ahern D, Geha RS. Expression of the CD40 ligand in T lymphocytes and induction of IgE isotype switching. *Int Arch Allergy Imm* 1995; 107: 43-4.
17. Steinitz M, Klein G, Koskimies S, Makel O. EB virus-induced B lymphocyte cell lines producing specific antibody. *Nature* 1977; 269: 420-2.
18. MacKenzie T, Dosch HM. Clonal and molecular characteristics of the human IgE-committed B cell subset. *J Exp Med* 1989; 169: 407-30.