J. Biochem. **119**, 1056-1061 (1996)

Histone Hyperacetylation Plays a Role in Augmentation of IL-4-Induced IgE Production in LPS-Stimulated Murine B-Lymphocytes by Sodium Butyrate

Itaru Yamamoto,[1] Tetsuhiko Matsunaga, Kei Sakata, Yuko Nakamura, Seiichi Doi, and Fumie Hanmyou

Department of Immunochemistry, Faculty of Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700

Received for publication, December 11, 1995

We previously reported the augmentation by sodium butyrate (NaBu) of IL-4-induced IgE production in LPS-stimulated murine B-lymphocytes. Histone deacetylase inhibition may be involved in the molecular mode of action of NaBu. Thus, this study was accomplished to examine the involvement of histone hyperacetylation in IL-4-induced class switching promotion by NaBu with a specific histone deacetylase inhibitor, trichostatin A (TSA). TSA was found to enhance IL-4-dependent IgE production in a concentration-dependent manner (0.3-30 nM) as did NaBu, although neither compound affected the IgE production in the absence of IL-4. The effect of combined addition of suboptimal concentrations of NaBu and TSA to the culture appeared to be additive, not synergistic. Moreover, the intense of enhancement of IgE production observed by addition of both compounds at optimal concentrations appeared to be equivalent to that obtained with each maximal concentration. Furthermore, enhancement of IgE production by TSA or NaBu was confirmed to be absolutely IL-4 dependent and was not due to the shift of kinetics. On the other hand, various cell cycle inhibitors such as caffeine and theophylline (G_1 or G_2-arrest), hydroxyurea (S-arrest), and colchicine (M-arrest), none of which have the ability to inhibit histone deacetylase, were shown to have no effect. These findings suggest that histone hyperacetylation plays an important regulatory role in the modification of IL-4-dependent class switching to Cε in LPS-stimulated murine B-cells by NaBu.

Key words: histone deacetylase, IgE, IL-4, sodium butyrate, trichostatin A.

Recently, allergic diseases such as food allergy and pollinosis have been rapidly increasing, and thus it is important to investigate environmental materials which are able to regulate or stimulate allergic responses. Immediate-type allergic diseases, such as atopic dermatitis, asthma, and rhinitis, are caused mainly by specific IgE antibodies to allergens and are occasionally serious in the case of fagopyrism and apisination (*1, 2*).

Extensive studies to establish therapies are being developed, and many reports describing IgE regulating factors or cytokines are available. For instance, the cytokine, interleukin-4 (IL-4) (*3, 4*) or IL-13 (*5*) produced by T-cells was shown to induce IgG4 and IgE switching in humans and IgG1 and IgE switching in mice both *in vitro* and *in vivo*, whereas transforming growth factor β (TGF-β) directs IgA switching (*6*). Besides cytokines, contact-mediated signals are required for B-cell proliferation and immunoglobulin production. Recently, the ligand for CD40, which is expressed on activated $CD4^+$ T cells, was shown to be one such membrane-associated molecule that acts as a co-stimulatory signal for IL-4-dependent IgE production by both murine and human B cells (*7*). Moreover, several cytokines, including IL-2 (*8*), IL-5 (*9*), IL-6 (*10*), IL-8 (*11*), IL-10 (*12*), IL-12 (*13*), interferon-α (IFN-α), IFN-γ (*14*), and tumor necrosis factor-α (TNF-α) (*15*), modulate IL-4-induced IgG4 and IgE synthesis in humans.

Butyric acid, a straight-chain saturated 4-carbon fatty acid, is produced from non-polysaccharides (dietary fiber) as a byproduct through digestion and fermentation by gastrointestinal bacteria such as *Clostridium* and *Eubacterium*, which are ordinarily present in ruminants; and its concentration in human colon is around 20 mmol/kg. It is well established that a large amount of ester forms of butyrate is contained in dairy products. Sodium butyrate (NaBu) has been found to have a variety of effects on the growth and differentiation of cultured animal cells (*16*). When NaBu at millimolar concentrations is added to the culture of some cell lines, it inhibits cell proliferation, arresting the cell cycle predominantly in the G_1 phase and sometimes in the G_2 phase. It also induces differentiation in human and murine tumor cell lines; that is, an increase of membranous antigens and enzymatic activities. These effects of NaBu may be associated with hyperacetylation of histone resulting from inhibition of histone deacetylase and a change in chromatin structure. NaBu treatment of some

[1] To whom correspondence should be addressed. Tel: +81-86-251-7960, Fax: +81-86-251-7962

Abbreviations: IL-4, interleukin-4; LPS, lipopolysaccharide; NaBu, sodium butyrate; TSA, trichostatin A; TGF-β, transforming growth factor-β; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid).

cell lines modulates various gene expressions. Recently, a promoter fragment necessary for the butyrate-dependent activation of chicken embryonic globin gene was found in murine erythroleukemia cells. Furthermore, NaBu-responsive element (BRE) was found in the human immunodeficiency virus type I long terminal repeat (*17*).

During studies of agents which affect IgE synthesis, we incidentally observed that NaBu significantly enhanced IL-4-induced IgG1 and IgE production by LPS-stimulated murine splenic cells in a dose-dependent manner between 30 and 300 μM, causing slightly inhibitory effects on IgM and IgG3 production, whereas NaBu alone was not effective (*18-20*). This stimulatory effect of NaBu was completely dependent on the presence of IL-4. It was shown to be due to an increase in the number of IgE-secreting cells in the stimulated cells, which was detected by enzyme-linked immunosorbent assay (ELISA) spot assay on day 7. These findings suggested that NaBu might enhance polyclonal IgE production with an increase in the number of IgE-secreting cells by affecting IL-4-induced class switching to Cε in LPS-stimulated splenic B-cells. Subsequent studies revealed that NaBu enhanced IL-4-induced IgE mRNA expression and isotype switching to Cε in LPS-stimulated highly purified murine splenic B cells by analyzing mature and germline Cε mRNA with reverse transcribed polymerase chain reaction (RT-PCR). Furthermore, we attempted to analyze the class switched immunoglobulin gene in IL-4-+ LPS-stimulated purified B-cells by use of the nested PCR technique, and this provided evidence that NaBu increased switch fragments containing recombined Sμ/Sε regions. However, the mode of action of NaBu on promotion of class switching to Cε remains unclear.

In the present study, we examined whether the inhibition of histone deacetylase might be associated with the promotion by NaBu of IL-4-dependent class switching to Cε in LPS-stimulated purified murine splenic B-lymphocytes by using a specific histone deacetylase inhibitor, trichostatin A (*21, 22*).

MATERIALS AND METHODS

Experimental Animals—Female BALB/c mice (6 weeks of age) were purchased from Charles River Japan (Kanagawa). These mice were used at 8 to 12 weeks of age.

Reagents—RPMI-1640 medium was purchased from ICN Biomedicals Japan (Tokyo). Eagle's MEM was from Nissui Pharmaceutical (Tokyo). FBS was from GIBCO BRL (Gaithersburg, MD). Rat anti-mouse IgE mAb (clone; LO-ME-2) was obtained from Experimental Immunology Unit (Brussels, Belgium). Biotinylated sheep anti-mouse IgE Ab was from The Binding Site (Birmingham, UK). Goat anti-mouse IgM, goat anti-mouse IgG, purified mouse IgM, and IgG were purchased from Organan Teknika (Durham, NC). Horseradish peroxidase (HRP) conjugated goat anti-mouse IgM and IgG were from Kirkegaad & Perry Lab. (Gaithersburg, MD). Sodium butyrate, caffeine, theophylline, hydroxyurea, colchicine, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), *N*-2-hydroxyethylpiperadine-*N*′ 2-ethane sulfonic acid (HEPES), *Escherichia coli* LPS, and BSA were purchased from Sigma Chemical (St. Louis, MO). Recombinant murine IL-4 was from Genzyme (Cambridge, MA). Trichostatin A was from Wako Pure Chemical (Osaka). Anti-Thy 1.2 antibody (clone; F7D5) was from Serotec (Oxford, England). HRP conjugated streptavidin was from Zymed Lab. (San Francisco, CA). Low toxic rabbit complement was from Cederlane Lab. (Ontario, Canada). Penicillin G was from Banyu Pharmaceutical (Tokyo). Streptomycin was from Meiji Seika (Tokyo).

Purification of Murine Splenic B Lymphocytes—Small dense B cells were prepared according to the methods of Kehry and Hudak (*23*). Briefly, spleen cells, after red blood cell lysis, were depleted of adherent cells by incubation on plastic petri dishes (Falcon 1005A, Falcon Labware, Oxnard, CA) in 10 mM HEPES (pH 7.2) buffered MEM supplemented with 10% heat-inactivated FBS at 37°C for 90 min, followed by antibody and complement treatment to remove T cells. This step was done at high cell density (2×10^7 cells/ml) with F7D5 (anti-Thy 1.2 Ab) at final dilution of 1 : 25 at 4°C for 30 min and low toxic rabbit complement treatment at final dilution of 1 : 15 at 37°C for 40 min, their live cells were counted. There was no proliferative response to Con A. FACS analysis also revealed that T-cells remaining in the treated culture cells were less than 3% (data not shown).

In Vitro IgE Production by IL-4-+LPS-Stimulated B-Cells—The *in vitro* culture was carried out as described by Snapper *et al.* (*24*) with some modifications. Briefly, 2.5×10^5 B-cells/ml were incubated in quadruplicate cultures with *Escherichia coli* LPS (10 μg/ml) and recombinant murine IL-4 (50 U/ml) and with or without various agents in round-bottom 96-well microplates (Nunc, Roskilde, Denmark) at 0.2 ml/well for various periods at 37°C under 5% CO_2 and 95% air. At fixed intervals, cell-free supernatants were collected and stored frozen at −20°C until immunoglobulin determinations were performed as described below. As a murine B-cell culture medium, we used RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 50 μM 2-ME, 100 U/ml penicillin G, and 100 μg/ml streptomycin.

Measurement of Immunoglobulins by ELISA—IgE was determined by ELISA as described by Azuma *et al.* (*25*) with some modifications. Briefly, flat-bottom 96-well multiplates (Corning Glass, Corning, NY) were coated with 50 μl of 4 μg/ml rat anti-mouse IgE mAb diluted with 15 mM PBS for 2 h at 37°C. After washing with PBS, plates were blocked with 100 μl of 2% BSA in PBS (BSA/PBS) for 2 h at 37°C. After washing, appropriately diluted culture supernatants with BSA/PBS and standard IgE (purified anti-TNP mAb) were added to anti-mouse IgE coated wells at 50 μl/well, and plates were incubated for 1 h at 37°C. After washing, 50 μl of 2.5 μg/ml biotinylated sheep anti-mouse IgE Ab diluted with BSA/PBS was added to each well, and plates were incubated for 1 h at 37°C. After washing extensively, 50 μl of 0.625 μg/ml HRP-conjugated streptavidin diluted with BSA/PBS was added to the wells, and plates were incubated for 1 h at 37°C. Again after washing, the peroxidase activity was assayed after adding 100 μl of 0.1 M citrate buffer (pH 4.0) containing 2.5 mM ABTS and 0.17% H_2O_2 followed by incubation at room temperature for 30 min. Absorbance at 405 nm was measured with an ELISA autoreader (Sanko Junyaku, Tokyo). The calibration curve was made using standard IgE.

IgM and IgG antibodies were measured as follows: the wells (MaxiSoap) (Nunc) were coated with 10 μg/ml of goat anti-mouse IgM Ab and 40 μg/ml of goat anti-mouse

IgG Ab, respectively, and after washing, plates were blocked with 100 μl of BSA/PBS for 2 h at 37°C. After washing, appropriate diluted culture supernatants with BSA/PBS and standard IgM or IgG were added to anti-mouse IgM or IgG coated wells at 50 μl/well, and incubated for 1 h at 37°C. After washing, HRP-conjugated goat anti-mouse IgM (100 ng/ml) or IgG (50 ng/ml) was added, and again after washing, the peroxidase activity was assayed after adding 100 μl of 0.1 M citrate buffer (pH 4.0) containing 2.5 mM ABTS and 0.17% H_2O_2 followed by incubation at room temperature for 30 min. Absorbance at 405 nm was measured, and the calibration curve was made using purified IgM or IgG. This ELISA system allowed the determination of as low concentration as 1 ng/ml of IgE, IgM, and IgG. All data were presented as mean value±SD of quadruplicate assays. Typical data from several repeated experiments are presented.

RESULTS

Effects of NaBu or TSA on IL-4-Induced IgE Synthesis by LPS-Stimulated Purified Murine B-Lymphocytes—We have confirmed that NaBu promotes IL-4-induced IgE synthesis by LPS-stimulated purified murine B-lymphocytes in a concentration-dependent manner ranging from 10 to 200 μM. Here, we examined whether histone deacetylase inhibition by NaBu was associated with its promotive activity of IgE production in IL-4- and LPS-stimulated B-lymphocytes, by use of a specific histone deacetylase inhibitor, TSA. This antibiotic like NaBu, was found to enhance the IgE production in a concentration-dependent manner ranging from 0.3 to 30 nM as shown in Fig. 1, although TSA did not enhance IgM and IgG productions (Fig. 2). TSA alone did not stimulate IgE production: namely, the effect of TSA was observed only in the presence of IL-4 (data not shown).

Time Course of the Effect of NaBu or TSA on IL-4-

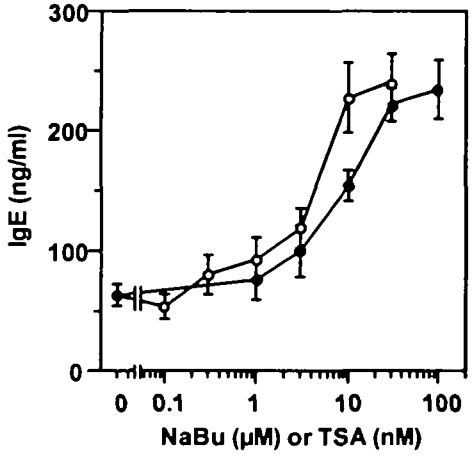

Fig. 1. **Effect of sodium butyrate (NaBu) or trichostatin A (TSA) on IL-4-induced IgE synthesis by LPS-stimulated purified murine B lymphocytes.** The cells (5×10^4 cells/200 μl/well) were cultured with or without varying concentrations of NaBu (●) or TSA (○) in the presence of LPS (10 μg/ml) and IL-4 (50 U/ml) for 7 days. After incubation, IgE levels in the conditioned medium were determined by ELISA. Results are expressed as mean value±SD for quadruplicate cultures.

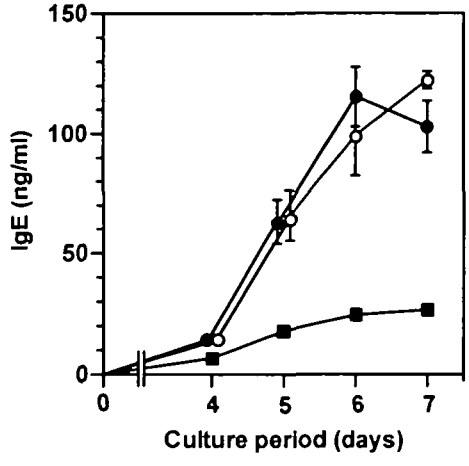

Fig. 3. **Time course of the effect of NaBu or TSA on IL-4-induced IgE production by LPS-stimulated murine splenic B lymphocytes.** The cells (5×10^4 cells/200 μl/well) were cultured with compounds (none, ■; NaBu, 100 μM, ●; TSA, 3 μM, ○) in the presence of LPS (10 μg/ml) and IL-4 (50 U/ml). The conditioned media were harvested at times indicated and IgE levels were determined by ELISA. Results are expressed as mean value±SD for quadruplicate cultures.

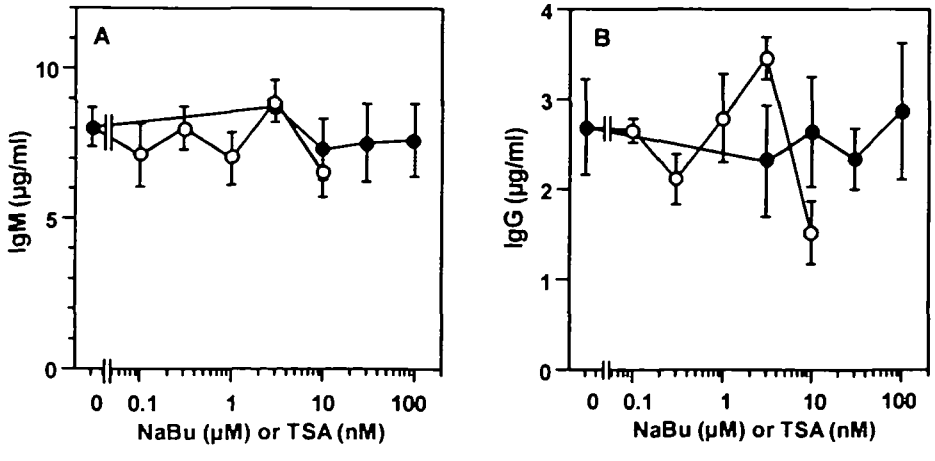

Fig. 2. **Effects of NaBu or TSA on IL-4-induced IgM and IgG synthesis by LPS-stimulated purified murine B-lymphocytes.** The cells (5×10^4 cells/200 μl/well) were cultured with or without varying concentrations of NaBu (●) or TSA (○) in the presence of LPS (10 μg/ml) and IL-4 (50 U/ml) for 7 days. After incubation, IgM (A) and IgG (B) levels in the conditioned medium were determined by ELISA. Results are expressed as mean value±SD for quadruplicate cultures.

Induced IgE Production by LPS-Stimulated Murine Splenic B-Lymphocytes—We examined the possibility that the enhancement of IL-4-induced IgE production by NaBu or TSA might be due to a shift of the kinetics by determining the kinetics of IgE production for 7 days after stimulation. It was found that neither NaBu nor TSA changed the kinetics of IgE production: namely, the enhancing activity was observed during the experimental period after day 4 (Fig. 3). It was also found that for enhancement of IgE production, NaBu or TSA should be present in the culture medium at a relatively early period after IL-4 and LPS stimulation (Fig. 4).

Effects of Simultaneous Additions of NaBu and TSA on IL-4-Induced IgE Production by LPS-Stimulated B-Cells—To determine the correlation between histone deacetylase inhibition and augmentation of IgE synthesis, we examined the effects of simultaneous additions of NaBu and TSA on IL-4-induced IgE production by LPS-stimulated B-cells. For this purpose, suboptimal concentrations (10 μM and 1 nM) and optimal concentrations (100 μM and 10 nM) of NaBu and TSA, respectively, were used. Combined addition of the suboptimal concentrations of each compound resulted in enhancement of IgE production to a comparable level to that observed with the optimal concentration of either compound. Moreover, addition of both compounds at optimal concentration showed limited enhancement of IgE production which was equivalent to that obtained with each optimal concentration (Fig. 5).

Effects of Cell Cycle Inhibitors on IL-4-Induced IgE Production by LPS-Stimulated B-Lymphocytes—NaBu and TSA are characterized as histone deacetylase inhibitors. They can block cell cycles at G_1/G_2 phase and are occasionally used as reagents synchronized culture (*26*). In this experiment, we examined whether cell cycle inhibitors such as caffeine and theophylline (G_1 phase blocker), hydroxyurea (S phase blocker) and colchicine (M phase blocker) would promote the IgE synthesis. It was found that IgE synthesis in IL-4−+LPS-stimulated B-cells was not influenced by the cell cycle inhibitors examined (Fig. 6). These inhibitors were also confirmed not to enhance IgM and IgG synthesis (data not shown).

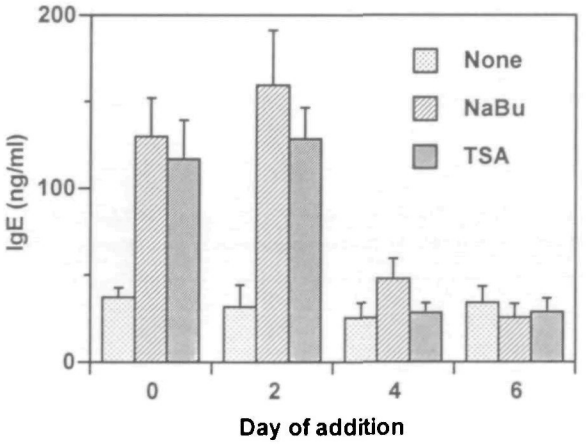

Fig. 4. **Effect of addition of NaBu or TSA at different times of culture on IL-4-induced IgE production by LPS-stimulated murine splenic B lymphocytes.** The cells (5×10^4 cells/200 μl/well) were cultured in the presence of LPS (10 μg/ml) and IL-4 (50 U/ml) for 7 days. Medium, NaBu (100 μM) or TSA (3 nM) was added at the time indicated. The conditioned medium was harvested at day 7 and IgE level was determined by ELISA. Results are expressed as mean value±SD for quadruplicate cultures.

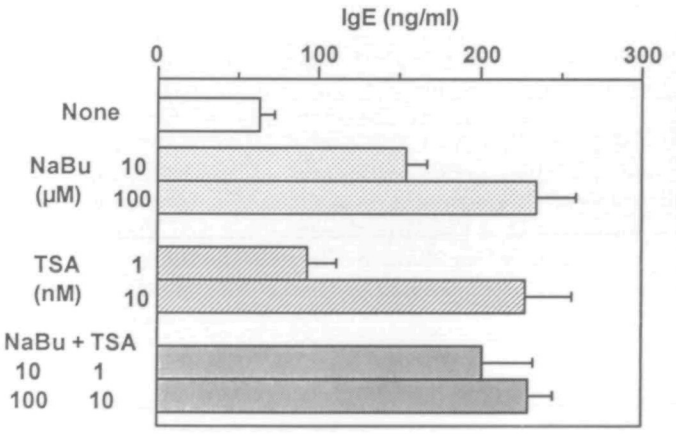

Fig. 5. **Effects of combined additions of NaBu and TSA on IL-4-induced IgE production by LPS-stimulated B cells.** The cells (5×10^4 cells/200 μl/well) were cultured with or without NaBu and/or TSA in the presence of LPS (10 μg/ml) and IL-4 (50 U/ml) for 7 days. After incubation, IgE levels in the conditioned medium were determined by ELISA. Results are expressed as mean value±SD for quadruplicate cultures.

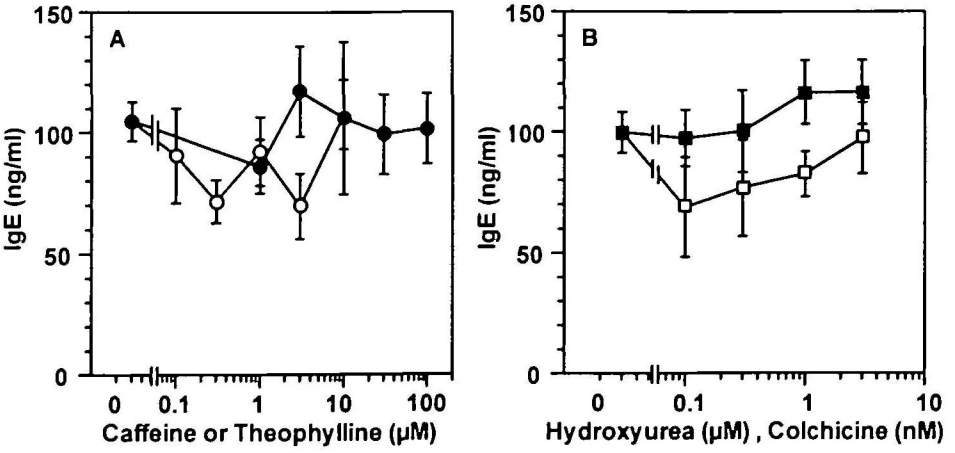

Fig. 6. **Effects of cell cycle inhibitors on IL-4-induced IgE production by LPS-stimulated B-lymphocytes.** The cells (5×10^4 cells/200 μl/well) were cultured with or without cell cycle inhibitors (theophylline, ○; caffeine, ●; hydroxyurea, ■; colchicine, □) at varying concentrations in the presence of LPS (10 μg/ml) and IL-4 (50 U/ml) for 7 days. After incubation, IgE levels in the conditioned medium were determined by ELISA. Results are expressed as mean value±SD for quadruplicate cultures.

DISCUSSION

We previously reported that NaBu promoted IL-4-induced IgE production by LPS-stimulated murine splenic B-lymphocytes and this enhancement by NaBu was completely dependent on the existence of IL-4. Subsequent studies indicated that this effect is attributed to the enhancement of immunoglobulin class switching to Cε (in preparation). However, the mode of action of NaBu on promotion of class switching to Cε remains unclear.

NaBu is reported to have diverse biological activities including inhibition of histone deacetylase, regulation of gene expression such as *c-fos* and protein synthesis such as estrogen receptor, and inhibition of cell proliferations (*27*). In the present study, we examined whether inhibition of histone deacetylase might contribute to the mode of action of NaBu in terms of class switch modification, because hyper-acetylation is believed to cause activation of various gene expressions (*28, 29*).

The IgE production was assessed by use of a specific inhibitor of histone deacetylase, TSA, which was originally described as an antibiotic for fungi. This antibiotic was found to enhance the IgE production in LPS-+IL-4-stimulated B-lymphocytes in a concentration-dependent manner, ranging from 0.3 to 30 nM, which is less than 1/10,000 of that by NaBu for promotion of IgE synthesis as shown in Fig. 1. Previously, we observed that NaBu augmented the IgE production at all concentrations of IL-4 tested. However, a strict requirement of IL-4 for NaBu effect was observed, because no IgE production was detected in the absence of IL-4 (*18-20*). We observed that TSA alone had no effect on IgE production. The inhibition constants (K_i) of NaBu and TSA for histone deacetylase are reported as 60 μM (*28*) and 3.4 nM (*22*), respectively, which correlate well with their abilities to promote IgE production. These results suggest that augmentation of IL-4-induced IgE synthesis in LPS-stimulated B-lymphocytes is associated with histone hyperacetylation due to inhibition of this enzyme by NaBu.

To determine the correlation between histone deacetylase inhibition and augmentation of IgE synthesis, we examined the effects of combined additions of NaBu and TSA on IL-4-induced IgE productions by LPS-stimulated B-cells. For this purpose, their suboptimal and optimal concentrations were used. As a result, the combined addition of suboptimal concentrations of NaBu and TSA was found to have additive, not synergetic, effects in terms of enhancement of the IgE production. Moreover, additions of both compounds at optimal concentrations showed the enhancement of IgE production which did not exceed that obtained with each optimal concentration (Fig. 3). These results suggest a close correlation between the enhancement of IgE production and the inhibition of histone deacetylase by NaBu.

Next, we examined whether cell cycle inhibitors other than NaBu and TSA, none of which are able to inhibit histone deacetylase, would modify IL-4-induced IgE production. These cell cycle inhibitors included caffeine and theophylline which are able to raise intracellular cyclic AMP contents by inhibiting phosphodiesterase, hydroxyurea which inhibits ribonucleoside-diphosphate or triphosphate reductase, and colchicine which inhibits microtuble polymerization; but none affected IgE production over a wide range of concentrations. These results together indicate that augmentation of IgE synthesis by NaBu is directly related to histone deacetylase inhibitory activity, but not cell cycle blocking.

Acetylation of histone in the chromatin is regulated at a constant level by two enzymes, acetyltransferase and histone deacetylase (*28, 29*). It is believed that histone deacetylase inhibition by NaBu would cause hyperacetylation, which would induce changes in an electron charge and/or steric conformation of the chromatin, followed by changes in the binding of transcriptional factors or RNA polymerase. A gene reconstitution enzyme called recombinase is considered to play an important role in IL-4-induced class switch to Cε (*30, 31*). It is expected that the approach of this enzyme to the specific switch regions on the antibody genes would be facilitated by the presence of NaBu.

Our experiments have demonstrated that NaBu enhances IL-4-induced polyclonal IgE production, but not the antigen-specific ongoing IgE production (*18-20*). These findings suggest that NaBu, an abundant ingredient in dairy products, may be a useful in immunotherapy for IgE-dependent allergy, since the large amount of polyclonal IgE induced would be able to dilute the antigen-specific IgE, thereby suppressing symptoms of atopic diseases.

REFERENCES

1. Vercelli, D. and Geha, R.S. (1991) Regulation of IgE synthesis in humans: A tale of two signals. *J. Allergy Clin. Immunol.* **88**, 285-297
2. Geha, R.S. (1992) Regulation of IgE synthesis in humans. *J. Allergy Clin. Immunol.* **90**, 143-150
3. Paul, W.E. (1991) Interleukin-4: Prototypic immunoregulatory lymphokine. *Blood* **77**, 1859-1870
4. de Vries, J.E., Gauchat, J.-F., Aversa, G.G., Punnonen, J., Gascan, H., and Yssel, G. (1991) Regulation of IgE synthesis by cytokines. *Curr. Opin. Immunol.* **3**, 851-858
5. McKenzie, A.N.J., Culpepper, J.A., de Waal Malefyt, R., Brière, F., Punnonen, J., Aversa, G., Sato, A., Dang, W., Cocks, B.G., Menon, S., de Vries, J.E., Banchereau, J., and Zurawski, G. (1993) Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function. *Proc. Natl. Acad. Sci. USA* **90**, 3735 3739
6. Kim, P.H. and Kagnoff, M.F. (1990) Transforming growth factor β1 increases IgA isotype switching at the clonal level. *J. Immunol.* **145**, 3773-3778
7. Armitage, R.J., Fanslow, W.C., Strockbine, L., Sato, T.A., Clifford, K.N., Macduff, B.M., Anderson, D.M., Gimpet, S.D., Davis-Smith, T., Maliszewski, C.R., Clark, E.A., Smith, C.A., Grabstein, K.H., Cosman, D., and Spriggs, M.K. (1992) Molecular and biological characterization of a murine ligand for CD40. *Nature* **357**, 80-82
8. Miyajima, H., Hirano, T., Hirose, S., Karasuyama, H., Okumura, K., and Ovary, Z. (1991) Suppression by IL-2 of IgE production by B cells stimulated by IL-4. *J. Immunol.* **146**, 457-462
9. Pène, J., Rousset, F., Brière, F., Chrétien, I., Wideman, J., Bonnefoy, J.Y., and de Vries, J.E. (1988) Interleukin 5 enhances interleukin 4-induced IgE production by normal human B cells. The role of soluble CD23 antigen. *Eur. J. Immunol.* **18**, 929-935
10. Vercelli, D., Jabara, H.H., Arai, K., Yokota, T., and Geha, R.S. (1989) Endogenous interleukin 6 plays an obligatory role in interleukin 4-dependent human IgE synthesis. *Eur. J. Immunol.* **19**, 1419-1424
11. Kimata, H., Yoshida, A., Ishioka, C., Lindley, I., and Mikawa, H. (1992) Interleukin 8 (IL-8) selectively inhibits immunoglobulin

E production induced by IL-4 in human B cells. *J. Exp. Med.* **176**, 1227-1231
12. Punnonen, J., de Waal-Malefyt, R., van Vlasselaer, P., Gauchat, J.-F., and de Vries, J.E. (1993) IL-10 and viral IL-10 prevent IL-4-induced IgE synthesis by inhibiting the accessory cell function of monocytes. *J. Immunol.* **151**, 1280-1289
13. Kiniwa, M., Gately, M., Gubler, U., Chizzonite, R., Fargeas, C., and Delespesse, G. (1992) Recombinant interleukin-12 suppresses the synthesis of immunoglobulin E by interleukin-4 stimulated human lymphocytes. *J. Clin. Invest.* **90**, 262-266
14. Pène, J., Rousset, F., Brière, F., Chrétien, I., Bonnefoy, J.-Y., Spits, H., Yokota, T., Arai, N., Arai, K., Banchereau, J., and de Vries, J.E. (1988) IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons γ and α and prostaglandin E2. *Proc. Natl. Acad. Sci. USA* **85**, 6880-6884
15. Gauchat, J.-F., Aversa, G., Gascan, H., and de Vries, J.E. (1992) Modulation of IL-4 induced germline ϵ RNA synthesis in human B cells by tumor necrosis factor-α, anti-CD40 monoclonal antibodies or transforming growth factor-β correlates with levels of IgE production. *Int. Immunol.* **4**, 397-406
16. D'Anna, J.A., Tobey, R.A., and Gurley, L.R. (1980) Concentration-dependent effects of sodium butyrate in chinese hamster cells: Cell-cycle progression, inner-histone acetylation, histone H1 dephosphorylation, and induction of an H1-like protein. *Biochemistry* **19**, 2656-2671
17. Bohan, C.A., Robinson, R.A., Luciw, P.A., and Srinivasan, A. (1989) Mutational analysis of sodium butyrate inducible elements in the human immunodeficiency virus type I long terminal repeat. *Virology* **172**, 573-583
18. Inoue, T., Kanto, M., Inokuma, K., Harada, Y., and Yamamoto, I. (1992) Enhancement of IL-4-induced IgE production by sodium butyrate. *Digestive Organs Immunol.* **27**, 46-51
19. Inokuma, K., Sakata, K., and Yamamoto, I. (1993) Study on enhancement by sodium butyrate of IL-4-induced IgE production in murine splenic cells (II). *Digestive Organs Immunol.* **28**, 27-32
20. Sakata, K., Matsunaga, T., Nakamura, Y., and Yamamoto, I. (1994) Target cells of sodium butyrate as an enhancing agent of IL-4-induced IgE production by LPS-stimulated murine splenocytes. *Digestive Organs Immunol.* **29**, 43-48
21. Tsuji, N., Kobayashi, M., Nagashima, K., Wakisaka, Y., and Koizumi, K. (1976) A new antifungal antibiotic, trichostatin. *J. Antibiot.* **29**, 1-6
22. Yoshida, M., Kijima, M., Akita, M., and Beppu, T. (1990) Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J. Biol. Chem.* **265**, 17174-17179
23. Kehry, M.R. and Hudak, S.A. (1989) Characterization of B-cell population bearing Fcϵ receptor II. *Cell. Immunol.* **118**, 504-515
24. Snapper, C.M., Finkelman, F.D., and Paul, W.E. (1988) Differential regulation of IgG1 and IgE synthesis by interleukin 4. *J. Exp. Med.* **167**, 183-196
25. Azuma, M., Hirano, T., Miyajima, H., Watanabe, N., Yagita, H., Enomoto, S., Furusawa, S., Ovary, Z., Kinashi, T., Honjo, T., and Okumura, K. (1987) Regulation of IgE production in SJA/9 and nude mice. Potentiation of IgE production by recombinant interleukin 4. *J. Immunol.* **139**, 2538-2544
26. Yoshida, M. and Beppu, T. (1988) Reversible arrest of proliferation of rat 3Y1 fibroblasts in both the G1 and G2 phases by trichostatin A. *Exp. Cell Res.* **177**, 122-131
27. Kruh, J. (1982) Effects of sodium butyrate, a new pharmacological agent, on cells in culture. *Mol. Cell. Biochem.* **42**, 65-82
28. Cousens, L.S., Gallwits, D., and Albert, B.M. (1979) Different accessibilities in chromatin to histone acetylase. *J. Biol. Chem.* **254**, 1716-1723
29. López-Rodas, G., Brosch, G., Georgieva, E.I., Sendra, R., Franco, L., and Loidl, P. (1993) Hypothesis. Histone deacetylase. A key enzyme for the binding of regulatory proteins to chromatin. *FEBS Lett.* **3**, 175-180
30. Yancopoulos, G.D., DePinho, R.A., Zimmerman, K.A., Lutzker, S.G., Rosenberg, N., and Alt, F. (1986) Secondary rearrangement events in pre-B cells: V_HDJ_H replacement by LINE-1 sequence and directed class switching. *EMBO J.* **5**, 3259-3266
31. Stavnezer-Nordgren, J. and Sirlin, S. (1986) Specificity of immunoglobulin heavy chain class switch correlates with activity of germline heavy chain genes prior to switching. *EMBO J.* **5**, 95-102