Histamine Selectively Enhances Human Immunoglobulin E (IgE) and IgG4 Production Induced by Anti-CD58 Monoclonal Antibody

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Summary

We studied the effects of histamine on human immunoglobulin (IgE) and IgG4 production. Histamine selectively enhanced IgE and IgG4 production in purified surface IgE and IgG4 negative ($sIgE^-sIgG4^-$) B cells from normal donors stimulated with interleukin (IL)-4 plus anti-CD58 or IL-13 plus anti-CD58 monoclonal antibody (mAb) without affecting production of IgG1, IgG2, IgG3, IgM, IgA1, or IgA2. In cultures with IL-4 plus anti-CD58 mAb, histamine-induced enhancement of IgE and IgG4 production was specifically blocked by thioperamide (H_3 receptor antagonist), and was inhibited by anti-IL-10 antibody (Ab). In contrast, in cultures with IL-13 plus anti-CD58 mAb, histamine-induced enhancement was blocked by dimaprit (H_1 receptor antagonist), and was inhibited by anti-IL-6 mAb. Histamine also enhanced IgE and IgG4 production by in vivo–generated $sIgE^+$ and $sIgG4^+$ B cells, respectively, from atopic patients; enhancement was blocked by dimaprit and thioperamide, and was inhibited by anti-IL-6 mAb and anti-IL-10 Ab. In $sIgE^-sIgG4^-$ B cells, IL-4 plus anti-CD58 mAb induced IL-10 production and IL-10 receptor expression, whereas IL-13 plus anti-CD58 mAb induced IL-6 production and IL-6 receptor expression. Histamine increased IL-10 and IL-6 production without affecting IL-10 and IL-6 receptor expression, in cultures with IL-4 plus anti-CD58 mAb and with IL-13 plus anti-CD58 mAb, respectively, which was blocked by thioperamide and dimaprit, respectively. In contrast, $sIgE^+$ and $sIgG4^+$ B cells spontaneously produced both IL-6 and IL-10 and constitutively expressed IL-6 and IL-10 receptors, and histamine increased IL-6 and IL-10 production without affecting IL-6 or IL-10 receptor expression, which was blocked by thioperamide and dimaprit. These results indicate that histamine enhanced IgE and IgG4 production by increasing endogenous IL-6 and IL-10 production via H_1 and H_3 receptors, respectively.

Many cytokines and factors are involved in human IgE and IgG4 production. IL-4 induces IgE and IgG4 production in purified B cells stimulated with anti-CD40 mAb, or hydrocortisone by isotype switching (1–5). IL-6, IL-10, and TNF-α enhance IL-4–induced IgE and IgG4 production in such cultures, whereas IL-8, and TGF-β inhibited IgE and IgG4 production (3, 6–9). In contrast, IL-5, IL-9, IL-12, IFN-α, and IFN-γ, which either enhance or inhibit IgE and IgG4 production induced by IL-4 in T cell–dependent culture, have no effect on IgE and IgG4 production in purified B cells (9–12). These results indicate that there are various IgE- and IgG4-modulatory factors in IL-4–stimulated cultures. Recently, in addition to IL-4, IL-13 has been shown to induce IgE and IgG4 production in purified B cells stimulated with anti-CD40 mAb (13, 14). We have reported that IL-13 induces IgE and IgG4 production in purified B cells stimulated with hydrocortisone (9, 15). Moreover, anti-CD58 mAb was also found to induce IgE production in IL-4–stimulated cultures (16). However, the effects of various cytokines on IgE and IgG4 production induced by IL-4 plus anti-CD58 mAb or by IL-13 plus anti-CD58 mAb have not been studied in depth.

Histamine is an autocoid released from mast cells and basophils by IgE- and IgG4-dependent stimulation (17, 18). It has been reported that histamine modulates production of cytokines by various cells, and that it enhances production of IL-6 and IL-8 by B and endothelial cells, respectively, whereas it inhibits TNF-α production by monocytes (19–21). In addition, histamine inhibits human IgG and IgM production in purified B cells stimulated with *Sta-*

phylococcus aureus Cowan strain I plus IL-2, or in mononuclear cells stimulated with pokeweed mitogen (22, 23). In contrast, histamines were found to either inhibit or enhance IgG or IgM production in human B cell lines (22, 24). These findings indicate that histamine differentially modulates Ig production depending on stimuli of target B cells. Since the effect of histamine on IgE and IgG4 production has not yet been reported, we examined the effect of histamine on their production in human B cells stimulated with IL-4 plus anti-CD58 mAb, or with IL-13 plus anti-CD58 mAb. Modulation of the effect of histamine by various cytokines is also discussed.

Materials and Methods

Reagents. Histamine dihydrochloride (HIS)[1], diphenhydramine (DIP, H_1 receptor antagonist), dimaprit (DIM, H_2 receptor agonist), cimetidine (CIM, H_2 receptor antagonist), (R)-α-methylhistamine (RAM, H_3 receptor agonist), and thioperamide (THI, H_3 receptor antagonist) were purchased from Sigma Chemical Co. (St. Louis, MO). 2-methylhistamine (MET, H_1 receptor agonist) was kindly provided by Smith Kline & French (Philadelphia, PA). The following recombinant human cytokines and Abs were kindly provided by companies noted previously (1, 9, 14): IL-4 and rabbit anti-IL-4 Ab (Ono Pharmaceutical Company, Osaka, Japan), IL-2 and IFN-α (Takeda Chemical Industries, Osaka), and growth hormone and rabbit anti–growth hormone Ab (Sumitomo Pharmaceutical Company, Osaka, Japan). Recombinant human IL-13 was purchased from Pepro Tech Inc. (Rocky Hill, NJ). Recombinant human IL-6, IL-9, IL-10, IL-12, IFN-γ, and TGF-β, and mouse IgG1 anti-IL-6 and goat anti-IL-10 Ab were purchased from R&D Systems, Inc. (Minneapolis, MN). Mouse IgM anti-CD40 mAb (BL-C4), mouse IgG2a anti-CD58 mAb (BRIC5), and rabbit anti-IL-13 Ab were purchased from Cosmo Bio Co. (Tokyo, Japan). The culture medium was DME, supplemented with Ham's Nutrient (DME/F-12; Sigma Chemical Co.), 0.5% BSA, and 50 μg/ml transferrin (9, 14).

Cell Cultures. Tonsillar mononuclear cells were obtained from nonatopic donors (serum IgE level <50 U/ml) and atopic patients (serum IgE level 1,578–12,259 IU/ml). Then, highly purified B cells were separated by SRBC rosetting, followed by L-leucine methyl ester incubation, as described previously (9, 14). Purified B cell fractions contained <1% $CD3^+$ T cells, <1% $CD14^+$ monocytes, <1% $CD16^+$ NK cells, and >98% $CD20^+$ B cells. Purified B cells were further separated into small resting B cells by Percoll density centrifugation, and small B cells were depleted of surface IgE–positive ($sIgE^+$) and $sIgG4^+$ B cells by panning (9, 14). The percentage of $sIgE^+$ and $sIgG4^+$ B cells was <0.1%. These $sIgE^-sIgG4^-$ B cells were cultured (2×10^5/0.2 ml/well) in U-bottomed microtiter plates (Costar Corp., Cambridge, MA) for 14 d in the presence or absence of various factors as described in Results. Alternatively, $sIgE^+$, $sIgE^-$, $sIgG4^+$, and $sIgG4^-$ B cells were purified from tonsillar B cells of atopic patients by panning. The percentages, respectively, of $sIgE^+$ and $sIgG4^-$ B cells in the $sIgE^-$ and $sIgG4^-$ B cell fractions were <0.1%. On the other hand, purified $sIgE^+$ and $sIgG4^+$ B cell fractions contained >98% $sIgE^+$ B cells and >98% $sIgG4^+$ B cells, respectively (9, 14). Purified $sIgE^+$, $sIgE^-$, $sIgG4^+$, and $sIgG4^-$ B cells were cultured (2×10^4/0.2 ml/well) for 14 d as described in Results. Control cultures for the evaluation of preformed Ig were carried out in the presence of cycloheximide (100 μg/ml). The amounts of IgE, IgG, IgM, and IgA subclasses in the supernatants were determined by ELISA (9, 15). In some experiments, $sIgE^-sIgG4^-$, $sIgE^+$, and $sIgG4^+$ B cells were cultured (2×10^5/0.2 ml/well) with various factors for 2 d, and the production of IL-6 and IL-10 was determined by ELISA (R&D Systems, Inc.). Simultaneously, the binding of IL-6, IL-10, (R)-α-methylhistamine, and diphenhydramine to those cells was studied by using biotinylated IL-6 and IL-10 (R&D Systems, Inc.), and biotinylated (R)-α-methylhistamine and diphenhydramine as previously reported (9). The mean fluorescence intensity (MFI) value of biotinylated factor–specific binding, determined after the subtraction of nonspecific binding in the presence of a 100-fold excess of unlabeled factors, was expressed as ΔMFI (9).

In Vivo Effect of Antihistamine on IgE and IgG4 Production in Atopic Patients. Four atopic patients (patients with atopic dermatitis, serum IgE level 2,344–5127 IU/ml, age 15–45-yr old) were treated with oral antihistamine (clemastine, 6 mg/d) and nonsteroidal anti-inflammatory ointment (bufexamac ointment). Alternatively, three atopic patients (patients with atopic dermatitis, serum IgE level 1,168–3685 IU/ml, age 16–32-yr old) were treated with bufexamac ointment alone. Peripheral blood was drawn before and after 2 wk of each treatment, and large B cells were purified as above. They were cultured (2×10^5/0.2 ml/well) with medium alone for 14 d, and the amount of IgE and IgG4 in the supernatants were determined by ELISA (9, 15).

Results and Discussion

Initial experiments have shown that histamine, either alone or with IL-4, IL-13, or anti-CD58 mAb, did not induce production of IgE (<0.2 ng/ml, $n = 10$) or IgG4 (<0.3 ng/ml, $n = 10$) by $sIgE^-sIgG4^-$ B cells. However, as shown in Fig. 1 *A*, histamine enhanced IgE and IgG4 production induced by IL-4 plus anti-CD58 mAb in a dose-dependent fashion. In contrast, histamine did not affect production of IgM, IgG1, or IgG2 (Fig. 1 *B*) or IgA1, IgG3, or IgA2 (Fig. 1 *C*). Identical results were observed in cultures stimulated with IL-13 plus anti-CD58 mAb. Histamine selectively enhanced production of IgE and IgG4 in a dose-dependent fashion (Fig. 1 *D*), without affecting production of IgM, IgG1, IgG2, IgG3, IgA1, or IgA2 (Fig. 1, *E* and *F*). In 10 experiments, histamine (10^{-5} M) enhanced production of IgE (264 ± 87% enhancement) and IgG4 (245 ± 92% enhancement) induced by IL-4 plus anti-CD58 mAb, while it enhanced production of IgE (378 ± 95% enhancement) and IgG4 (321 ± 102% enhancement) induced by IL-13 plus anti-CD58 mAb. In contrast, histamine did not enhance (<20% enhancement) production of IgM, IgG1, IgG2, IgG3, IgA1, or IgA2 in these cultures ($n = 10$).

It has been reported that histamine modulates Ig production or cytokine synthesis through one of the H_1, H_2, or H_3 receptors, or through more than one type of receptor (19–24). To study which receptors were involved in enhancement of IgE and IgG4 production, B cells were stimulated with histamine, and H_1, H_2, and H_3 receptor antagonist

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[1] *Abbreviations used in this paper:* CIM, cimetidine; DIM, dimaprit; DIP, diphenhydramine; HIS, histamine; MET, 2-methylhistamine; MFI, mean fluorescence intensity; RAM, (R)-α-methylhistamine; $sIgE^+$, surface IgE positive; THI, thioperamide.

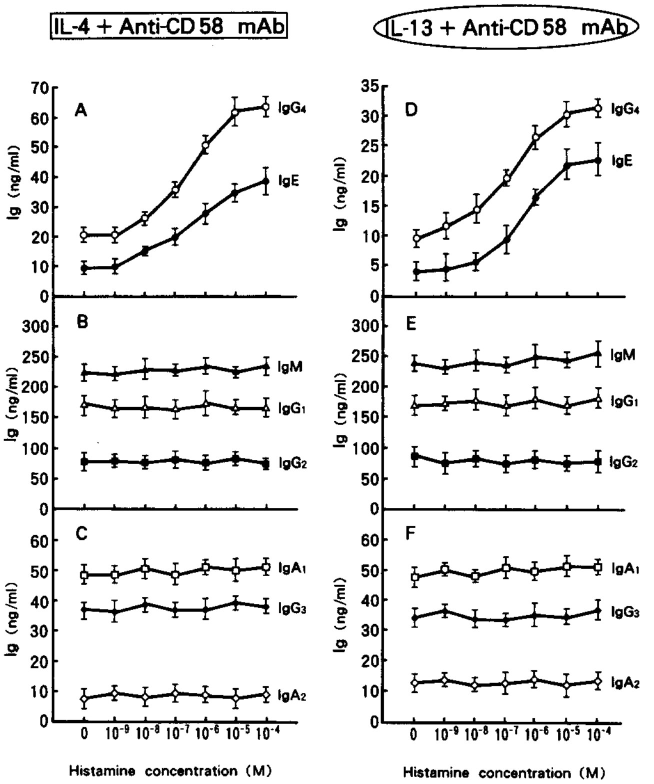

Figure 1. Effects of histamine on Ig production. Purified sIgE$^-$sIgG4$^-$ B cells from nonatopic donors were cultured (10^5/well) with IL-4 (1,000 U/ml) plus anti-CD58 mAb (1 μg/ml) (*A–C*), or IL-13 (500 ng/ml) plus anti-CD58 mAb (*D–F*) in the presence or absence of increasing concentrations of histamine. After 14 d of culture, production of IgE, IgG4 (*A* and *D*), IgM, IgG1, IgG2 (*B* and *E*), IgA1, IgG3, and IgA2 (*C* and *F*) was determined. Values are means ± 1 SD of triplicate cultures from one experiment, representative of ten.

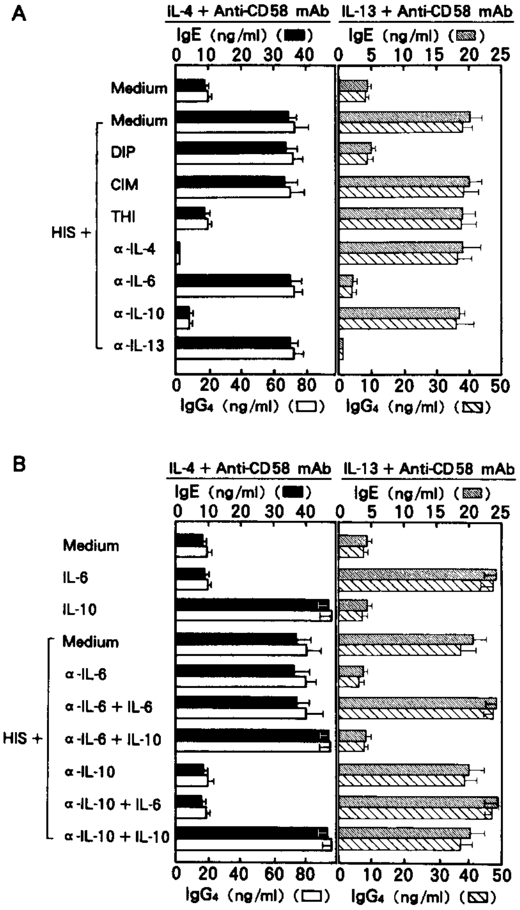

Figure 2. Effects of various factors on histamine-induced enhancement of IgE and IgG4 production. Purified sIgE$^-$sIgG4$^-$ B cells from nonatopic donors were cultured (10^5/well) with IL-4 (1,000 U/ml) plus anti-CD58 mAb (1 μg/ml) or IL-13 (500 ng/ml) plus anti-CD58 mAb in the presence or absence of the indicated factors. Histamine (*HIS*) was used at 10^{-5} M, diphenhydramine (DIP), cimetidine (*CIM*), and thioperamide (*THI*) each at 10^{-4} M, all the Abs at 10 μg/ml, and IL-6 and IL-10 each at 300 ng/ml. After 14 d of culture, IgE and IgG4 production were determined. Values are means ± 1 SD of triplicate cultures.

were added. As shown in Fig. 2 *A*, *left*, in cultures stimulated with IL-4 plus anti-CD58 mAb, histamine-induced IgE and IgG4 production was not affected by diphenhydramine (DIP, H_1 receptor antagonist) or cimetidine (CIM, H_2 receptor antagonist), whereas enhancement was completely blocked by thioperamide (THI, H_3 receptor antagonist).

We have previously reported that, in purified B cells, IL-4 plus hydrocortisone or IL-13 plus hydrocortisone induced IgE and IgG4 production, and this production was enhanced by IL-6 and IL-10. In contrast, other cytokines, IL-1β, IL-2, IL-3, IL-5, IL-7, IL-9, IL-11, or IL-12 did not enhance IgE or IgG4 production (9). In addition, nerve growth factor or growth hormone, which enhanced IgE and/or IgG4 production (1, 13), were without effect (data not shown). It is thus possible that histamine-induced enhancement was due to production of IL-4, IL-6, IL-10, or IL-13. As shown in Fig. 2 *A*, *left*, in cultures stimulated with IL-4 plus anti-CD58 mAb, IgE and IgG4 production was completely abrogated by anti-IL-4 mAb. In addition, histamine-induced enhancement was inhibited by anti-IL-10 Ab, whereas neither anti-IL-6 mAb nor anti-IL-13 Ab did so. In contrast, in cultures stimulated with IL-13 plus anti-CD58 mAb, histamine-induced enhancement of IgE and IgG4 production was blocked by diphenhydramine, but not by cimetidine or thioperamide. Moreover, anti-IL-6 mAb, but not anti-IL-4 mAb or anti-IL-10 Ab, inhibited histamine-induced enhancement, and anti-IL-13 Ab abrogated IgE and IgG4 production (Fig. 2 *A*, *right*).

We then studied the specificity of the effects of IL-6 and IL-10. As shown in Fig. 2 *B*, *left*, in cultures stimulated with IL-4 plus anti-CD58 mAb, addition of IL-10, but not IL-6, enhanced IgE and IgG4 production. Histamine-induced enhancement was blocked by anti-IL-10 Ab, which was reversed by high concentrations of IL-10, but not by IL-6. In contrast, in cultures stimulated with IL-13 plus anti-CD58 mAb, IL-6 enhanced IgE and IgG4 production. Anti-IL-6 blocked histamine-induced enhancement, which was reversed by IL-6, but not by IL-10. Collectively, these results indicated that in cultures stimulated with IL-4 plus anti-CD58 mAb, histamine enhanced IgE and IgG4 production through H_3 receptors and by involvement of IL-10, but not of IL-6. This is in accordance with the previous report by others (16) that IL-6 did not affect IgE production induced by IL-4 plus anti-CD58 mAb. In contrast, in cultures stimulated with IL-13 plus anti-CD58 mAb, histamine enhanced IgE and IgG4 production through H_1 receptors and by involvement of IL-6.

It is possible that histamine enhanced IgE and IgG4 production by affecting isotype switching of $sIgE^-$ and $sIgG4^-$ B cells, respectively, or alternatively, by directly affecting $sIgE^+$ and $sIgG4^+$ B cells, respectively. To test this, we took advantage of the fact that in vivo-generated $sIgE^+$ and $sIgG4^+$, but not $sIgE^-$ and $sIgG4^-$ B cells obtained from atopic patients spontaneously produced IgE and IgG4, respectively (1, 14, 15). As shown in Fig. 3 *A*, Expt. 1, histamine enhanced IgE production by $sIgE^+$ B cells, which was blocked by diphenhydramine and thioperamide, whereas cimetidine and IFN-γ failed to do so. Other inhibitory cytokines, IL-12, IFN-α, TGF-β, and Abs to stimulatory cytokines, including antigrowth hormone, antinerve growth factor, and anti-IL-9 Abs were also without effect (data not shown). However, histamine failed to induce IgE production by $sIgE^-$ B cells (Fig. 3 *A*, Expt. 1). On the other hand, spontaneous IgE production by $sIgE^+$ B cells was not inhibited by anti-IL-4 mAb or anti-IL-13 Ab, whereas it was inhibited by anti-IL-6 mAb and anti-IL-10 Ab. Moreover, anti-IL-6 mAb and anti-IL-10 Ab each inhibited histamine-induced enhancement of IgE production (Fig. 3 *A*, Expt. 2). Furthermore, simultaneous addition of anti-IL-6 mAb and anti-IL-10 Ab almost completely inhibited spontaneous and histamine-induced IgE production by $sIgE^+$ B cells. IL-6 or IL-10 each partially reversed inhibition by anti-IL-6 mAb plus anti-IL-10 Ab. However, addition of both IL-6 and IL-10 completely reversed inhibition of IgE production. In contrast, histamine with these factors failed to induce IgE production by $sIgE^-$ B cells (Fig. 3 *A*, Expt. 3). Moreover, histamine with IL-4, IL-13, or anti-CD58 mAb did not induce IgE production by $sIgE^-$ B cells (<0.2 ng/ml) as observed by $sIgE^-$ B cells from nonatopic donors.

As shown in Fig. 3 *B*, Expts. 1–3, identical results were observed for IgG4 production by $sIgG4^+$ and $sIgG4^-$ B cells. Spontaneous IgG4 production by $sIgG4^+$ B cells was enhanced by histamine, IL-6, and IL-10, whereas it was inhibited by anti-IL-6 mAb or anti-IL-10 Ab, but not by anti-IL-4 mAb or anti-IL-13 Ab. Histamine-induced enhancement was blocked by diphenhydramine and thioperamide, but not by dimaprit, and was inhibited by anti-IL-6 mAb and anti-IL-10 Ab. Addition of both IL-6 and IL-10 reversed inhibition by anti-IL-6 mAb plus anti-IL-10 Ab, whereas histamine with these factors failed to induce IgG4 production by $sIgG4^-$ B cells (Fig. 3, Expt. 3). Histamine with IL-4, IL-13, or with anti-CD58 mAb also failed to induce IgG4 production by $sIgG4^-$ B cells (<0.3 ng/ml). Taken together, these results indicated that histamine enhanced IgE and IgG4 production by directly affecting $sIgE^+$ and $sIgG4^+$ B cells, respectively, via both H_1 and H_3 receptors and by involvement of IL-6 and IL-10.

It is possible that histamine-induced enhancement of IgE and IgG4 production was due to the increase in IL-6 and/

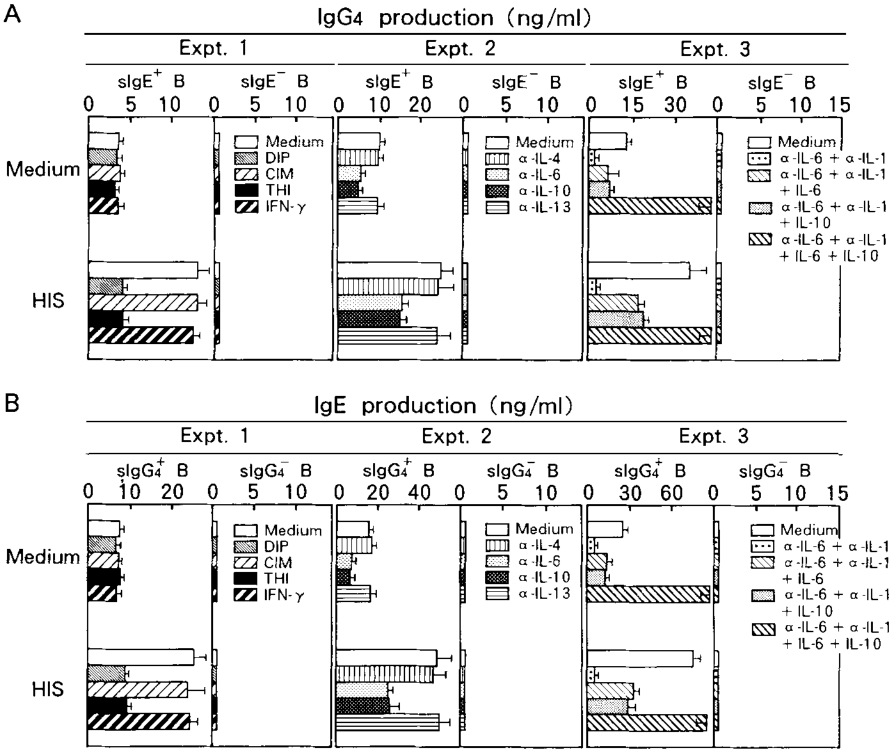

Figure 3. Effects of histamine on IgE and IgG4 production by sIg^+ and sIg^- B cells from atopic patients. Purified $sIgE^+$ or $sIgE^-$ (*A*), and $sIgG4^+$ or $sIgG4^-$ (*B*) B cells were cultured (2 × 10^4/well) with indicated factors. Histamine (*HIS*) was used at 10^{-5} M, diphenhydramine (DIP), cimetidine (*CIM*), and thioperamide (*THI*) each at 10^{-4} M, IFN-γ at 1,000 U/ml, IL-6 and IL-10 each at 300 ng/ml, and all the Abs at 10 μg/ml. After 14 d of culture, the production of IgE (*A*) and IgG4 (*B*) was determined. Values are means ± 1 SD of triplicate cultures.

or IL-10 production, or alternatively, due to the enhanced expression of IL-6 and/or IL-10 receptors, in sIgE$^+$ and sIgG4$^+$ B cells, respectively. To study this possibility directly, we stimulated sIgE$^-$sIgG4$^-$ B cells from nonatopic donors with IL-4 plus anti-CD58 mAb, or with IL-13 plus anti-CD58 mAb. Alternatively, sIgE$^+$ and sIgG4$^+$ B cells from atopic patients were cultured with medium. These cells were incubated with histamine in the presence or absence of histamine receptor antagonists or agonists, and production of IL-6 and IL-10, and IL-10 receptor expression were determined. In sIgE$^-$sIgG4$^-$ B cells, medium alone did not induce production of IL-6 or IL-10 ($<$0.01 ng/ml), or expression of IL-6 or IL-10 receptors. However, as shown in Fig. 4 *A*, IL-4 plus anti-CD58 mAb induced IL-10 production and IL-10 receptor expression, whereas it failed to induce IL-6 production or IL-6 receptor expression. Histamine enhanced IL-10 production without affecting IL-10 receptor expression, whereas histamine failed to induce IL-6 production or IL-6 receptor expression. Histamine-induced enhancement of IL-10 production was blocked by thioperamide, but not by dimaprit. Moreover, whereas 2-methylhistamine (MET, H_1 receptor agonist) or dimaprit (DIM, H_2 receptor agonist) failed to enhance IL-10 production, (R)-α-methylhistamine (RAM, H_3 receptor agonist) did enhance production. On the other hand, IL-13 plus anti-CD58 mAb induced IL-6 production and IL-6 receptor expression, while having no effect on IL-10 production or IL-10 receptor expression. Histamine enhanced IL-6 production without affecting IL-6 receptor expression, and enhancement was blocked by diphenhydramine, but not by thioperamide. In addition, (R)-α-methylhistamine enhanced IL-6 production, whereas 2-methylhistamine or dimaprit failed to do so (Fig. 4 *B*). In contrast, as shown in Fig. 4, *C* and *D*, sIgE$^+$ and sIgG4$^+$ B cells from atopic patients spontaneously produced both IL-6 and IL-10, and expressed IL-6 and IL-10 receptor. Histamine enhanced production of IL-6 and IL-10 without affecting receptor expression, which was specifically blocked by diphenhydramine and thioperamide, respectively. Moreover, 2-methylhistamine and (R)-α-methylhistamine, respectively, enhanced production of IL-6 and IL-10 without affecting IL-6 and IL-10 receptor expression. Taken together, enhancement of IgE and IgG4 production by histamine was due to the increase in production of IL-6 and/or IL-10, but not due to the increased expression of IL-6 and IL-10 receptors.

In these studies, we have demonstrated that histamine selectively enhanced IgE and IgG4 production. However, the mechanisms of the histamine-induced enhancement differed depending on the culture systems used. In cultures of sIgE$^-$sIgG4$^-$ B cells stimulated with IL-4 plus anti-CD58 mAb, histamine-induced enhancement was due to the increased production of IL-10 through H_3 receptors. This is not surprising. It has been reported that, in addition to functional presynaptic autoreceptors in the brain cortex, H_3 receptors were involved in IL-8 secretion by endothelial cells, contractions of the ileum, and corticosterone secretion by the adrenal cortex (20, 25, 26). To the best of our knowledge, this is the first report that H_3 receptors were involved in IL-10 production, resulting in enhancement of IgE and IgG4 production. In accordance with this, as shown in Fig. 5, although H_3 receptors were not expressed on freshly separated sIgE$^-$sIgG4$^-$ B cells ($<$3 ΔMFI), they were induced by IL-4 plus anti-CD58 mAb, but not by IL-13 plus anti-CD58 mAb ($<$3 ΔMFI). In contrast, H_3 receptors were constitutively expressed on in vivo-generated sIgE$^+$ and sIgG4$^+$ B cells.

On the other hand, in cultures stimulated with IL-13 plus anti-CD58 mAb, histamine enhanced IgE and IgG4 production through increased production of IL-6 via H_1

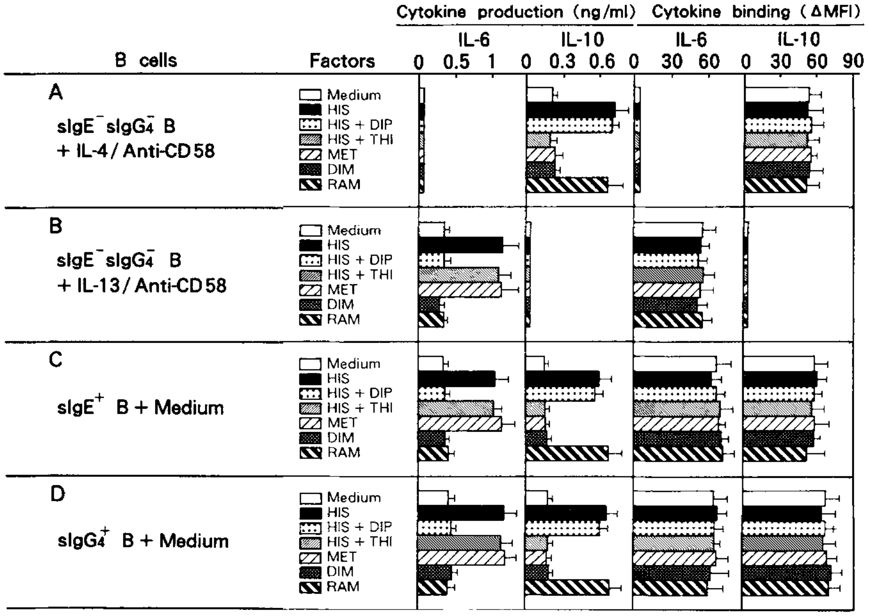

Figure 4. Effects of histamine or histamine receptor agonists on the production and the binding of IL-6 and IL-10. Purified sIgE$^-$sIgG4$^-$ B cells from nonatopic donors were cultured with IL-4 plus anti-CD58 mAb (*A*), or with IL-13 plus anti-CD58 mAb (*B*), whereas sIgE$^+$ (*C*) and sIgG4$^+$ (*D*) B cells from atopic patients were cultured with medium or indicated factors. Histamine was used at 10^{-5} M, other factors at 10^{-4} M. After 2 d of culture, the production and the binding of IL-6 and IL-10 were determined. Values are means ± 1 SD of triplicate cultures.

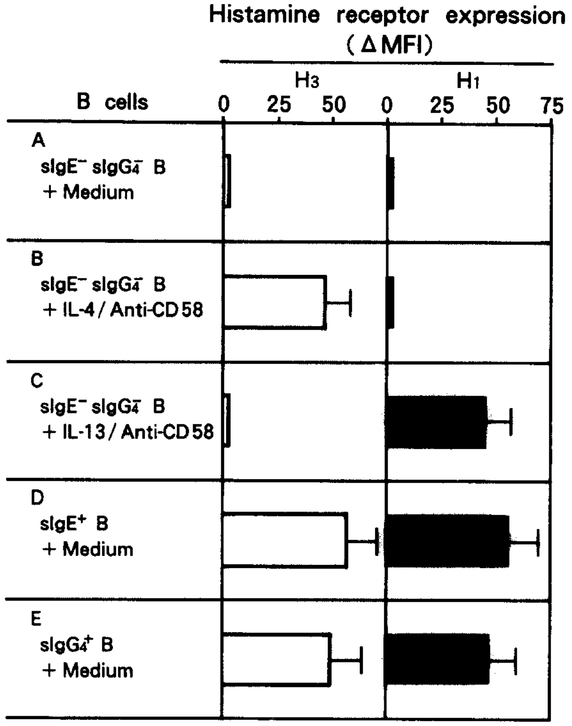

Figure 5. Histamine receptor expression on various cells. Purified sIgE$^-$sIgG4$^-$ B cells from nonatopic donors were cultured with medium (*A*), IL-4 plus anti-CD58 mAb (*A*), or with IL-13 plus anti-CD58 mAb (*B*), whereas sIgE$^+$ (*C*) and sIgG4$^+$ (*D*) B cells from atopic patients were cultured with medium. After 2 d of culture, the binding of (R)-α-methylhistamine (H_3 receptor) and diphenhydramine (H_1 receptor) was determined. Values are the means ± 1 SD of four experiments.

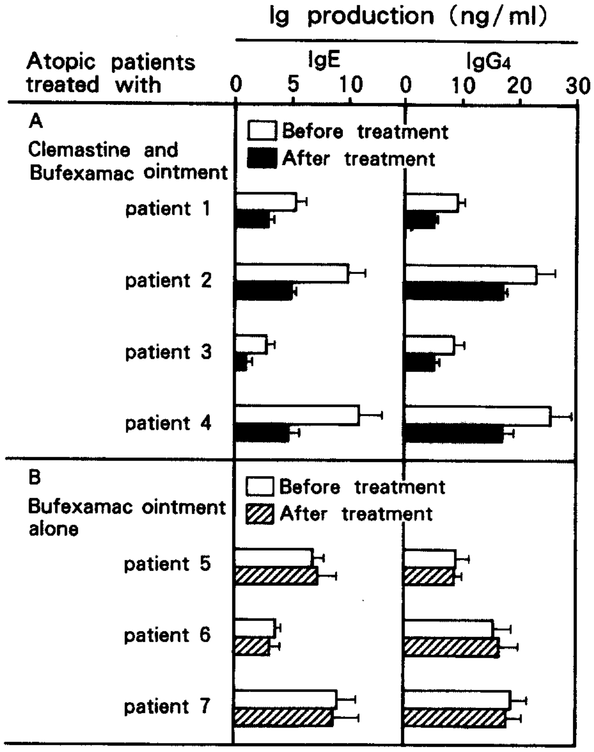

Figure 6. Effect of oral antihistamine on IgE and IgG4 production in atopic patients. Patients with atopic dermatitis were treated with oral antihistamine (*clemastine*) and nonsteroidal anti-inflammatory ointment (*bufexamac*) (*patients 1–4*) (*A*) or with bufexamac ointment alone (*patients 5–7*) (*B*). Peripheral blood B cells from patients before and after each treatment were cultured wtih medium alone. After 14 d of culture, IgE and IgG4 production was determined. Values are the means ± 1 SD of triplicate cultures.

receptors. This is in accordance with the report that (*a*) IL-13 increased IL-6 production by human keratinocytes, and (*b*) histamine enhanced IgM production through increased production of IL-6 via H_1 receptors in B cells (24, 27). Moreover, as shown in Fig. 5, H_1 receptors were not expressed on sIgE$^-$sIgG4$^-$ B cells (<3 ΔMFI), however, they were induced by IL-13 plus anti-CD58 mAb, but not by IL-4 plus anti-CD58 mAb (<3 ΔMFI). In contrast, H_1 receptors were expressed constitutively on in vivo-generated sIgE$^+$ and sIgG4$^+$ B cells.

Taken together, these results suggested that in vivo IgE and IgG4 production was regulated by both IL-6 and IL-10, whereas in vitro IgE and IgG4 production induced by IL-13 or IL-4 plus anti-CD58 mAb was regulated by IL-6 or IL-10 independently. Although we and others have reported that IL-6 and IL-10 enhance in vitro IgE and IgG4 production in various culture systems (3, 5, 6, 9), the direct evidence of the effect of IL-6 and IL-10 on in vivo IgE and IgG production in humans has not been previously reported. However, IL-6 is released locally in antigen-challenged sites in atopic patients (28). IL-10 is overexpressed in the skin of patients with atopic dermatitis (29). It is possible that in atopic patients, production of IL-6 and IL-10 is increased at the local site, which may enhance IgE and IgG4 production there, thus resulting in aggravating IgE- and IgG4-mediated allergic reactions (18). This possibility is currently under investigation.

The fact that histamine enhanced IgE and IgG4 production stimulated with IL-4 or IL-13 is important in relation to the treatment of allergic diseases. It has been reported that IL-4 increased histamine release from basophils (30). In addition, we have found that IL-13 (500 ng/ml) also increases histamine release from human basophils (21 ± 6%, $n = 4$). Conversely, IgE and IgG4 were also involved in histamine release and IL-4 production (18, 31). Therefore, it is tempting to speculate that, in atopic patients, IL-4, IL-13 and histamine may produce a vicious circle. Treatment with antihistamines may decrease IgE and IgG4 production, resulting in improvement of allergy. Therefore, we studied the effect of oralantihistamine on IgE and IgG4 production in atopic patients. As shown in Fig. 6, spontaneous IgE production by B cells from atopic patients treated with oral H_1 receptor antagonist, clemastine (and nonsteroidal anti-inflammatory ointment, bufexamac) for 14 d, was decreased after treatment. Similarly, spontaneous IgG4 production by B cells from atopic patients decreased after treatment with clemastine (Fig. 6 *A*). In contrast, IgE and IgG4 production by B cells from atopic patients treated with bufexamac ointment alone, as controls, was not decreased after treatment. These results indicate that histamine may affect IgE and IgG4 production in vivo. Studies on the detailed mechanisms as well as molecular analysis for the effect of histamine are currently in progress. Finally, histamine seems to be an excellent reagent for the study of IgE and IgG4 production.

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References

1. Kimata, H., and H. Mikawa. 1993. Nedocromil sodium selectively inhibits IgE and IgG4 production in human B cells stimulated with IL-4. *J. Immunol.* 151:6723–6734.
2. Jabara, H.H., S.M. Fu, R.S. Geha, and D.H. Vercelli. 1990. CD40 and IgE: synergism between anti-CD40 monoclonal antibody and interleukin 4 in the induction of IgE synthesis by highly purified human B cells. *J. Exp. Med.* 172:1861–1864.
3. Zhang, K., E.A., Clark, and A. Saxon. 1991. CD40 stimulation stimulation provides an IFN-γ–independent and IL-4–dependent differentiation signal directly to human B cells for IgE production. *J. Immunol.* 146:1836–1842.
4. Gascan, H., J.-F., Gauchat, G. Aversa, P. van Vlasseleaer, and J. E. de Vries. 1991. Anti-CD 40 monoclonal antibodies or $CD4^+$ T cell clones and IL-4 induce IgG4 and IgE switching in purified human B cells via different signaling pathways. *J. Immunol.* 147:8–13.
5. Jabara, H.H., D.J. Ahern, D. Vercelli, and R.F. Geha. 1991. Hydrocortisone and IL-4 induce IgE isotype switching in human B cells. *J. Immunol.* 147:1557–1560.
6. Rousset, F., E. Garcia, T. Defrance, C. Péronne, N. Vezzio, D.-H., Hsu, R. Kastelein, K.W. Moore, and J. Banchereau. 1992. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 89:1890–1893.
7. Gauchat, J.-F., G. Aversa, H., Gascan, and J.E. de Vries. 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.
8. Kimata, H., A. Yoshida, C. Ishioka, I. Lindley, and H. Mikawa. 1992. Interleukin 8 (IL-8) selectively inhibits immunoglobulin E production induced by IL-4 in human B cells. *J. Exp. Med.* 176:1227–1231.
9. Kimata, H. 1995. Differential effects of gangliosides on human IgE and IgG4 production. *Eur. J. Immunol.* 25:302–305.
10. Kiniwa, M., M. Gately, U. Gubler, R. Chizzonite, C. Fargas, and G. Delespesse. 1992. Recombinant interleukin-12 suppresses the synthesis of immunoglobulin E by interleukin-4 stimulated human lymphocytes. *J. Clin. Invest.* 90:262–266.
11. Kimata, H., A. Yoshida, C. Ishioka, and H. Mikawa. 1992. Differential effect of vasoactive intestinal peptide, somatostatin, and substance P on human IgE and IgG subclass production. *Cell. Immunol.* 144:429–442.
12. Dugas, B., J.C. Renauld, J. Pène, J.Y. Bonnefoy, P. Braquet, J.V. Snick, and J.M. Mencia-Heurta. 1993. Inerleukin-9 potentiates the interleukin-4–induced immunoglobulin (IgG, IgM and IgE) production by normal human B cells. *Eur. J. Immunol.* 23:1687–1692.
13. Aversa, G., J. Punnonen, B.G. Cocks, R. de Waal Malefyt, F. Vega, Jr., S.M. Zurawski, G. Zurawski, and J.E. de Vries. 1993. An interleukin 4 (IL-4) mutant protein inhibits both IL-4 or IL-13–induced human immunoglobulin G4 (IgG4) and IgE synthesis and B cell proliferation: support for a common component shared by IL-4 and IL-13 receptors. *J. Exp. Med.* 178:2213–2218.
14. Kimata, H., and M. Fujimoto. 1994. Growth hormone and insulin-like growth factor I induce immunoglobulin (Ig)E and IgG4 production by human B cells. *J. Exp. Med.* 180: 727–732.
15. Kimata, H., I. Lindley, and K. Furusho. 1995. Effect of hydrocortisone on spontaneous IgE and IgG4 production in atopic patients. *J. Immunol.* 154:3557–3566.
16. Diaz-Sanchez, D., S. Chegini, K. Chang, and A. Saxon. 1994. CD58 (LFA-3) stimulation provides a signal for human isotype switching and IgE production distinct from CD40. *J. Immunol.* 153:10–19.
17. Schleimer, R.P., C.P. Derse, B. Friedman, S. Gillis, M. Plaut, L.M. Lichtenstein, and D.W. MacGlashan, Jr. 1989. Regulation of human basophil mediator release by cytokines. I. Interaction with antiinflammatory steroids. *J. Immunol.* 143: 1310–1317.
18. Beauvais, F., C. Hieblot, and J. Benveniste. 1989. Effect of sodium cromoglycate and nedocromil sodium on anti-IgE–induced and anti-IgG4–induced basophil degranulation. *Drugs.* 37(Suppl. 1):4–8.
19. Falus, A., and K. Merétey. 1992. Histamine: an early messenger in inflammatory and immune reactions. *Immunol. Today.* 13:154–156.
20. Jeannin, P., Y. Delneste, P. Gosset, S. Molet, P. Lassalle, O. Hamid, A. Tsicopoulos, and A.B. Tonnel. 1994. Histamine induces interleukin-8 secretion by endothelial cells. *Blood.* 84:2229–2233.
21. Vannier, E., L.C. Miller, and C.A. Dinarello. 1991. Hista-

mine suppresses gene expression and synthesis of tumor necrosis factor α via histamine H_2 receptors. *J. Exp. Med.* 174: 281–284.

22. Fujimoto, M., and H. Kimata. 1994. Histamine inhibits immunoglobulin production via histamine H_2 receptors without affecting cell growth in human B cells. *Clin. Immunol. Immunopathol.* 73:96–102.
23. Lima, M., and R.E. Rocklin. 1981. Histamine modulates in vitro IgG production by pokeweed mitogen–stimulated human mononuclear cells. *Cell. Immunol.* 64:324–336.
24. Falus. A. 1993. Interleukin-6 biosynthesis is increased by hiatamine in human B-cell and glioblastoma cell lines. *Immunologgy.* 78:193–196.
25. Menkveld, G.J., and T. Hendrik. 1990. Inhibition of electrically evoked contractions of guinea-pig ileum preparations mediated by the histamine H_3 receptor. *Eur. J. Pharmacol.* 186:343–347.
26. LaBellea, F.S., G. Queen, G. Glavin, G. Durant, D. Stien, and L.J. Brandes. 1992. H_3 receptor anatgonist, thioperamide, inhibits adrenal steroidogenesis and histamine binding to adrenocortical microsomes and binds to cytochrome P450. *Br. J. Pharmacol.* 107:161–164.
27. Derocq, J.-M., M. Segui, C. Point-Chazel, A. Minty, D. Caput, P. Ferrara, and P. Casellas. 1994. Interleukin-13 stimulates interleukin-6 production by human keratinocytes. Similarity with interleukin-4. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 343:32–36.
28. Lee, C.E., M.E. Neuland, H.G. Teafold, B.F. Villacis, P.S. Dixon, S. Valtier, C.-H., Yeh., D.C. Fournier, and E.V. Charlesworh. 1992. Interelukin-6 is released in the cutaneous response to allergen challenge in atopic individuals. *J. Allergy Clin. Immunol.* 89:1010–1020.
29. Ohmen, J.D., J.M. Hanifin, B.J. Nickoloff, T.H. Rea, R. Wyzykowski, J. Kim, D. Jullien, T. McHugh, A. Nassif, S.C. Chan, and R.L. Modlin. 1995. Overexpression of IL-10 in atopic dermatitis. Contrasting cytokine pattern with delayed-type hypersensitivity reactions. *J. Immunol.* 154:1956–1963.
30. White, M.V., Y. Igarashi, B.E. Emery, M.T. Lotze, and M.A. Kaliner. 1992. Effects of in vivo administration of interleukin-2 (IL-2) and IL-4, alone and in combination, on ex vitro human basophil histamine release. *Blood.* 79:1491–1495.
31. Brunner, T., C.H. Heusser, and C.A. Dahinden. 1993. Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. *J. Exp. Med.* 177:605–612.