

Interferon (IFN)- α , IFN- γ , interleukin (IL)-2, and arachidonic acid metabolites modulate IL-4-induced IgE synthesis similarly in healthy persons and in atopic dermatitis patients

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The role of cytokines and arachidonic acid metabolites in the regulation of IgE production in healthy persons and in atopic dermatitis patients with elevated IgE levels was studied. Interleukin-4 (IL-4) induced IgE production in peripheral blood mononuclear cells (PBMCs) of all donors, and no significant difference was found between the amounts of IgE produced by healthy persons and atopic dermatitis patients. Similarly, recombinant interferon (IFN)- α and IFN- γ , as well as IL-2, inhibited IL-4-induced IgE production to a similar extent in both study groups. To evaluate the role of arachidonic acid (AA) metabolites in the regulation of IgE production, we added indomethacin, an inhibitor of the cyclooxygenase pathway, or nordihydroguaiaretic acid (NDGA), an inhibitor of the lipoxygenase pathway, to IL-4-treated cultures. Both indomethacin and NDGA strongly inhibited IL-4-induced IgE production. They also inhibited IL-4-induced IgG4 synthesis. No significant difference in the amount of inhibition was found between the two study groups. We were unable to restore the NDGA-induced inhibition of IgE-production by adding leukotrienes B₄, C₄, D₄, or 5-HETE to the NDGA-treated cultures. PGE₂ also failed to restore the indomethacin-mediated inhibitory effect. Consequently, NDGA- and indomethacin-mediated inhibitory effects do not appear to be mediated by any single factor studied. Collectively, our results show IFNs and IL-2 to be similar in effect in the modulation of IL-4-induced IgE synthesis in healthy and atopic persons. In addition, our results show the importance of AA metabolites in the regulation of IgE and IgG4 synthesis in normal persons as well as in atopic dermatitis patients.

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Isotype switching to IgE is a complex cascade of events, in which cytokines play a crucial role. Induction of IgE synthesis is dependent on interleukin (IL)-4 and an additional signal provided by CD4⁺ T cells (7, 19, 23). Despite the fact that IL-4 is the sole inducer of IgE synthesis, several other cytokines, such as IL-6, tumor necrosis factor (TNF)- α , transforming growth factor (TGF)- β , interferon (IFN)- α , and IFN- γ , are known to modulate IL-4-induced IgE synthesis in mature B cells (7, 19, 23, 33). In addition to induction of ϵ switching, IL-4 has also been found to induce switching to IgG4 (8, 13).

The reason for increased production of IgE in atopic patients is unclear. It has been reported that B cells of healthy and atopic persons have different signal requirements for induction of IgE synthesis (31). Recently, Th1- and Th2-type human T-cell clones were reported (18, 35). This finding seems to have important implications also in the regulation of IgE synthesis and in the pathogenesis of allergy, since allergen-specific T-cell clones isolated from atopic patients generally produce more IL-4 and less IFN- γ than do bacteria-specific T-cell clones (18, 24). The production of IFN- γ has been reported to be reduced also in patients with hyper-IgE syndrome (6)

and in atopic dermatitis patients (22). However, a disturbed balance in the production of IL-4 and IFN- γ is unlikely to be the only explanation for increased IgE synthesis in atopic patients, and it is not known why allergen-specific T cells produce more IL-4 than do other T cells.

In addition to cytokines, arachidonic acid (AA) metabolites are another group of molecules which may play a role in the regulation of B-cell differentiation and, hence, in IgE synthesis. AA can be derived from inositol phospholipids, from membrane phosphatidylcholine and phosphatidylethanolamine, or from external cellular sources (16). Free AA can be metabolized either into the cyclooxygenase pathway or into lipoxygenase products (27). Lipoxygenation of free AA leads to the formation of hydroperoxyeicosatetraenoic acids (HPETE) or more stable hydroxyeicosatetraenoic acids (HETA). 5-HPETE is further transformed to leukotrienes (LT) B₄, C, D, and E (27). On the other hand, the cyclooxygenase pathway leads to the formation of prostaglandins, the inhibition of which is the mechanism of action for aspirin-like drugs (32). Recent evidence suggests that AA metabolites are, in addition to their other effects, important regulators of B-lymphocyte function (9, 19, 25, 34), and PGE₂ has been reported to augment IgE production without affecting the production of other isotypes (17).

The aim of the present study was to investigate whether B cells of healthy and atopic persons respond differently to signals mediated by cytokines or AA metabolites. IL-4 was used to trigger IgE responses, and the effects of IL-2, IFN- α , and IFN- γ , and cyclooxygenase and lipoxygenase pathway inhibitors on IgE synthesis were studied. Our results show that healthy persons and atopic dermatitis patients are equally sensitive to IFN- and IL-2-mediated inhibition of IL-4-induced IgE synthesis. In addition, our results show that AA metabolites, the products of the lipoxygenase and cyclooxygenase pathways, play an important role in the modulation of IL-4-induced IgE production in healthy persons as well as in patients with atopic dermatitis.

Patients and methods

Patients and cell separations

Peripheral blood samples were obtained from healthy volunteers with no known history of atopic diseases and from adult patients who had a history of atopic dermatitis from early childhood. The diagnosis was made according to the criteria of Hanifin & Rajka (10), which are based on the typical distribution and appearance of the lesions. The female/male ratio of the patients was 1:3, and the age distribution was 25–44 years (mean 33 years). The mean IgE level in the patient sera was 12 000 IU/ml (range 2000–

35 000 IU/ml). In the control group the female/male ratio was 1:1, and the age distribution was 21–35 years (mean 26 years).

Mononuclear cells were isolated by Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient centrifugation. The cells were washed twice and resuspended in Iscove's Modified Dulbecco's Medium (IMDM) (Sigma) supplemented with 10% FCS (Gibco, Grand Island, NY, USA), and gentamycin (50 μ g/ml).

Reagents

Recombinant human IL-4 was obtained from Genzyme (Boston, MA, USA). Recombinant IL-2, IFN- α -2a, and IFN- γ were kindly provided by Hoffmann-La Roche, Inc. (Nutley, NJ, USA). PGE₂, LTB₄, LTC₄, LTD₄ and 5-HETE were purchased from Sigma Chemical Company (St Louis, MO, USA). Indomethacin was provided by Farnos Group LTD (Turku, Finland). NDGA was purchased from Aldrich Chemical Company (Milwaukee, WI, USA).

Cell cultures

Cells were cultured in IMDM, supplemented with 10% FCS and gentamycin (50 μ g/ml) in round-bottomed, 5-ml culture tubes (Nunc, Roskilde, Denmark) at a density of 10⁶/ml. The final culture volume was always 0.5 ml. The cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂. Ig levels in the culture supernatants were measured after 7 (IgG and IgM) or 14 (IgG4 and IgE) d of culture. All measurements were done in duplicate.

Because ethanol was used in primary dilution of AA metabolites, a control with 0.05% ethanol was combined with every experiment. This amount exceeded the amount of ethanol in any of the test cultures. The 0.05% ethanol was found to have no effect on IL-4-induced IgE or IgG4 synthesis (data not shown).

Measurement of Ig production

IgE levels were measured by ELISA according to the method described by Pene et al. (19). The sensitivity of IgE ELISA was 0.2 ng/ml. Since patients with high serum levels of IgE were studied, cell-associated IgE was extracted and measured in every experiment by brief acid treatment of freshly isolated peripheral blood mononuclear cells (PBMCs), according to the protocol described by Turner et al. (30). Acid treatment was also performed in the end of the cultures, and the level of IgE obtained from freshly isolated cells was subtracted from the final IgE concentration.

IgG4 secretion was determined by ELISA as described by Gascan et al. (8) with the following modifications. We coated 96-well plates (Costar, Cambridge, MA, USA), overnight with mouse mAbs against human IgG4 at 2 µg/ml (HP6023, The Binding Site, Birmingham, UK) in 125 mmol carbonate buffer, pH 9.6. The plates were washed three times in PBS containing 0.05% Tween 20, and saturated with 10% FCS in RPMI-1640 for 1 h at room temperature. The samples and standards (purified IgG4, Nordic Immunological Laboratories) were incubated for 6 h at 37°C. After washing, peroxidase labeled mAbs anti-κ and anti-λ (Tago, Inc., Burlingame, CA, USA) were added at a concentration of 1:25 000 in PBS with 0.1% bovine serum albumin (Sigma) and 0.05% Tween 20. After washing, bound enzyme activity was measured with 1 mg/ml of 2,2'-azinobis (3-ethylbenzothiazoline sulfonic acid) (Sigma) in 0.1 mol citrate-0.1 mol phosphate buffer, pH 4.5, containing 0.0003% H₂O₂ as substrate. The optical density (OD 405) was read in an ELISA reader. The sensitivity of the ELISA was 0.2 ng/ml.

The assay for the measurement of IgM and total IgG levels was totally automated by means of an AUTO-EIA benchtop analyzer (Labsystems, Helsinki), as described previously (20).

Statistics

The statistical significance was determined by two-tailed Student's *t*-test. A *P* value below 0.05 was considered to be significant.

Results

We first compared IL-4-induced IgE synthesis in atopic dermatitis patients with that in normal persons. PBMCs were cultured in the presence of increasing concentrations of recombinant IL-4, and the level of secreted IgE was measured by ELISA after 14 d of culture. IL-4 induced IgE production in the PBMCs of every donor (*n* = 16). IgE production was detectable in most cases at a concentration of 25 U/ml, but the highest production was observed at concentrations 100 and 200 U/ml (Fig. 1). The maximum response obtained varied remarkably from donor to donor. The lowest response was 1.5 ng/ml while the highest IgE level detected was 392 ng/ml. However, no significant difference in IL-4-induced IgE production was found when normal persons and atopic patients were compared, indicating that exogenous IL-4 can induce IgE synthesis in healthy persons as well as in atopic patients.

Because interferons (19) and IL-2 (14) have been reported to inhibit IL-4-induced IgE synthesis, we asked whether the increased IgE production in atopic patients was due to reduced sensitivity to

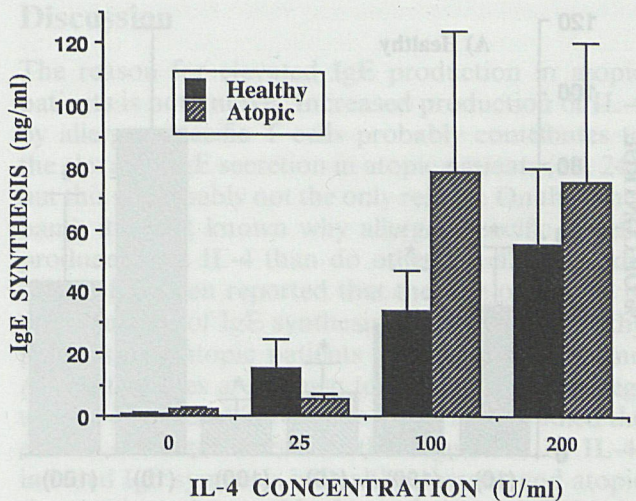


Fig. 1. IL-4 induces comparable IgE synthesis by PBMCs of healthy persons and atopic dermatitis patients *in vitro*. PBMCs were cultured in increasing concentrations of recombinant IL-4, and IgE levels in the culture supernatants were measured after 2 weeks. Eight persons were studied in both study groups, and each experiment was carried out in duplicate. The data represent the mean $\pm$ SE of IgE levels obtained.

cytokine-mediated inhibitory effects. Both IFNs, as well as IL-2, inhibited IL-4-induced IgE synthesis (Fig. 2). The inhibitory effect of IL-2 was significant (*P* < 0.05) only when used at a concentration of 100 U/ml. IFN-γ resulted in stronger inhibition than IFN-α or IL-2 (Fig. 2). However, no significant difference in the inhibition between healthy persons and atopic dermatitis patients was found. In other words, IL-2 and IFNs regulate IgE synthesis in healthy persons in the same way as in atopic patients.

To study the role of AA metabolites in the generation of IgE-producing cells, we treated IL-4-stimulated cultures with indomethacin and NDGA to inhibit the cyclooxygenase or lipoxygenase pathways, respectively. Indomethacin was used at a concentration of 0.75 µmol/l, which is known to result in a specific inhibition of the cyclooxygenase pathway (26). NDGA is known as a specific inhibitor of the lipoxygenase pathway (11, 26). Both indomethacin and NDGA strongly inhibited IL-4-induced IgE synthesis (Fig. 3A), but no significant difference in the degree of inhibition was detected between healthy and atopic persons.

Since IL-4 also induces switching to IgG4 (8, 13), we studied the effects of NDGA and indomethacin on IgG4 switching. No significant difference in the level of IL-4-induced IgG4 synthesis between the two groups was found (data not shown). As shown in Fig. 3B, 40–60% inhibition of IL-4-induced IgG4 synthesis by NDGA and indomethacin was detected. The degree of inhibition was similar in both groups.

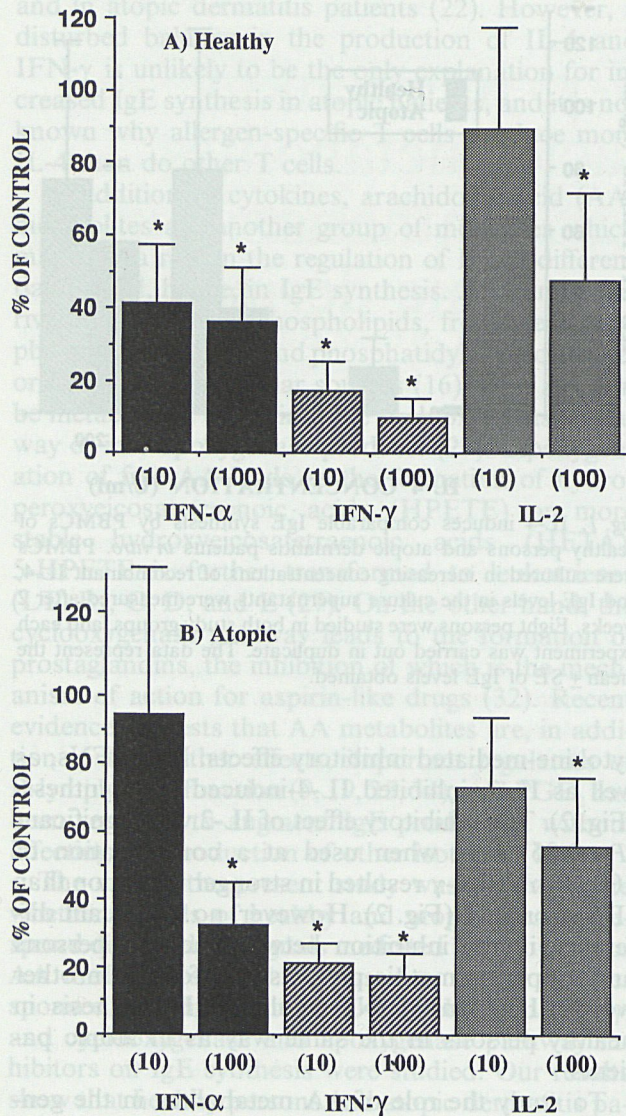


Fig. 2. IFN- α , IFN- γ , and IL-2 inhibit IgE synthesis in (A) healthy persons and (B) atopic dermatitis patients. PBMCs were cultured in recombinant IL-4 (100 U/ml), and IFN- α , IFN- γ , and IL-2 were added to the cultures at concentrations (U/ml) indicated in parentheses. Eight subjects were studied in both study groups. The mean $\pm$ SE of IL-4-induced IgE synthesis by PBMCs of atopic patients and healthy controls were 79 ± 45 ng/ml and 34 ± 13 ng/ml, respectively. Data are shown as % (mean $\pm$ SE) of IgE obtained in each experiment, as compared with those obtained by IL-4 only. Values with (*) are significantly ($P < 0.05$) different from control values.

To determine whether indomethacin and NDGA affect specifically IL-4-induced Ig isotype switching, or whether they act in B-cell differentiation in a more general way, we stimulated the cultures with a polyclonal B-cell activator, pokeweed mitogen (PWM), and indomethacin or NDGA was added. In PBMCs derived from healthy persons, both NDGA and indomethacin induced an inhibitory effect, the degree of which was, however, slightly lower than that of IL-4-induced IgG4 and IgE synthesis (Fig. 4). In

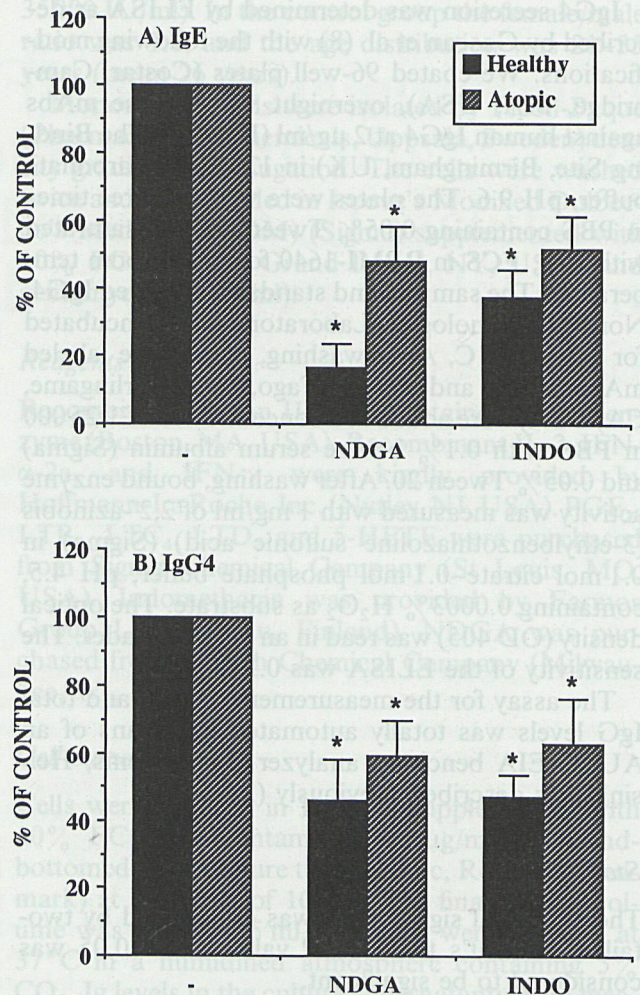


Fig. 3. Nordihydroguaiaretic acid (NDGA) and indomethacin (INDO) inhibit IL-4-induced production of (A) IgE and (B) IgG4 in healthy persons and in atopic dermatitis patients. PBMCs were cultured in recombinant IL-4 (100 U/ml) and NDGA (15 μ M) or INDO (0.75 μ M) were added to the cultures. Eight subjects were studied in both study groups. The mean $\pm$ SE of IL-4-induced IgG4 synthesis by PBMCs of atopic patients and healthy controls were 108 ± 49 ng/ml and 112 ± 51 ng/ml, respectively, and that of IL-4-induced IgE synthesis was as described in the Fig. 2 legend. Data are shown as % (mean $\pm$ SE) of IgE or IgG4 obtained in each experiment, as compared with those obtained by IL-4 only. Values with (*) are significantly ($P < 0.05$) different from control values.

PBMCs derived from atopic dermatitis patients, indomethacin failed to induce any inhibitory effect. NDGA, in contrast, inhibited both IgM and IgG synthesis (Fig. 4).

To study further the role of AA metabolites in IgE synthesis, we tried to restore the inhibitory effects of indomethacin and NDGA by adding AA metabolites to these cultures. PGE₂ was tested for restoration of indomethacin-induced inhibitory effect at concentrations 10^{-6} and 10^{-8} mol/l. However, PGE₂ failed to restore the inhibitory effect. For example, when PGE₂ was added at a concentration of

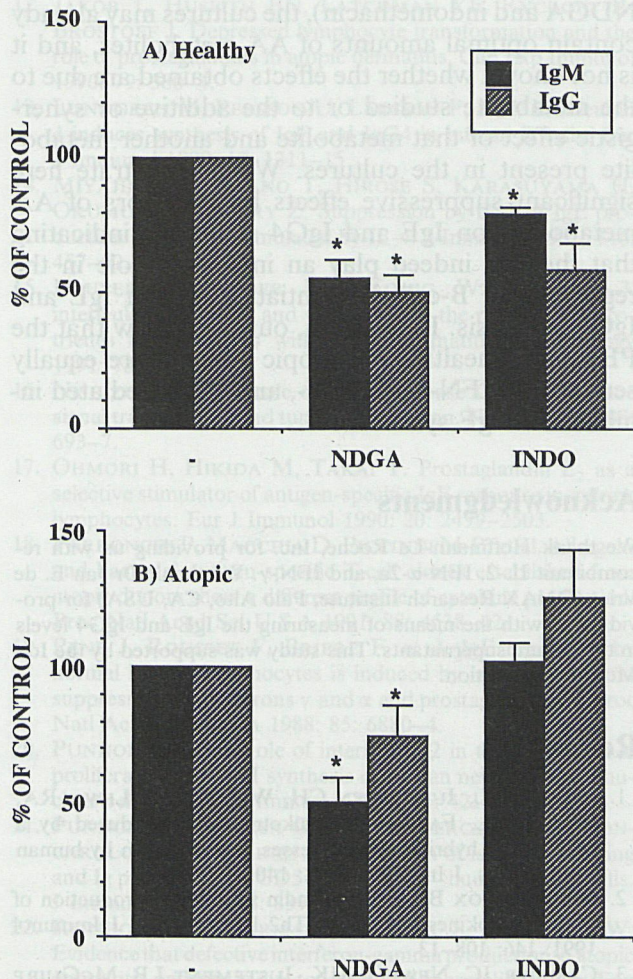


Fig. 4. The effects of nordihydroguaiaretic acid (NDGA, 15 μ mol) and indomethacin (INDO, 0.75 μ mol) on PWM-induced IgM and IgG synthesis in (A) healthy persons and in (B) atopic dermatitis patients. The mean $\pm$ SE of PWM-induced IgM and IgG synthesis by PBMCs of atopic patients ($n = 8$) were 1125 ± 275 ng/ml and 2072 ± 457 ng/ml, respectively. The mean $\pm$ SE of PWM-induced IgM and IgG synthesis by PBMCs of healthy persons ($n = 8$) were 4614 ± 1104 ng/ml and 4374 ± 1346 ng/ml, respectively. Data are shown as % (mean $\pm$ SE) of IgM and IgG obtained in each experiment, as compared with those obtained by PWM only. Values with (*) are significantly ($P < 0.05$) different from control values.

10^{-6} mol/l, the percentage of IgE obtained was $54.4 \pm 17.2\%$ (mean $\pm$ SE) of that obtained by IL-4 only, while it was $44.6 \pm 9.5\%$ without PGE₂. The lipoxygenase pathway products LTB₄, LTC₄, LTD₄, and 5-HETE were tested for restoration of the NDGA-mediated inhibitory effect at concentrations 10^{-8} and 10^{-10} mol/l. However, at both concentrations these factors failed to restore significantly the NDGA-mediated inhibition (data not shown). Consequently, neither the indomethacin- nor the NDGA-mediated inhibitory effect was due to inhibition of any single product tested in these experiments.

Discussion

The reason for elevated IgE production in atopic patients is not known. Increased production of IL-4 by allergen-specific T cells probably contributes to the elevated IgE secretion in atopic patients (18, 24), but this is probably not the only reason. On the other hand, it is not known why allergen-specific T cells produce more IL-4 than do other T cells. In addition, it has been reported that the role of T cells in the induction of IgE synthesis is different in healthy persons and atopic patients (31). IL-2, IFNs, and AA metabolites are known to play a role in the regulation of B-cell differentiation. We have studied the effects of these factors in the regulation of IL-4-induced IgE synthesis in healthy persons and atopic dermatitis patients with elevated serum IgE levels.

Our results show that the level of IL-4-induced IgE production *in vitro* is in the same range in healthy persons and atopic patients, a finding which is consistent with the results of Claassen et al. (5). These results indicate that the reason for elevated IgE production in atopic dermatitis patients is not increased sensitivity of their B cells to IL-4-mediated induction of IgE synthesis. This is also in line with recent results showing that even naive, unprimed B cells from fetal spleen, liver, or bone marrow can be induced to high levels of IgE production *in vitro* (21). In addition, because B cells of atopic dermatitis patients do not differ from other B cells in their capacity to produce IgE in response to IL-4, it seems obvious that the amount of IgE produced *in vivo* is dependent merely on the environment of the B cells rather than on the B cells themselves. We therefore studied the effects of other cytokines, such as IL-2 and IFNs, on IL-4-induced IgE synthesis. Recent studies show increased production of IL-4 and reduced production of IFN- γ by allergen-specific T cells in atopic patients (18, 24). We asked whether the PBMCs of atopic dermatitis patients are less sensitive to cytokine-mediated inhibition of IgE synthesis than the PBMCs of healthy persons. Significant inhibition of IL-4-induced IgE synthesis was observed in IFN- α , IFN- γ , and IL-2 at a concentration of 100 U/ml. However, the degree of inhibition was similar in healthy and atopic persons, indicating that the PBMCs of healthy and atopic persons are equally sensitive to inhibitory effects mediated by these cytokines. Consequently, the low level of IFN- γ -production in atopic patients may well contribute to the increased IgE synthesis in atopic patients.

Several previous studies suggest a role for AA metabolites in the pathophysiology of allergic diseases. Spontaneous leukotriene generation of neutrophils from patients with AD is higher, as compared with healthy persons (15). In addition, peripheral eosinophilic granulocytes from children

with asthma show enhanced leukotriene production (28). Moreover, atopic dermatitis patients show increased production of PGE₂ (12). However, studies on the effects of AA metabolites in the regulation of B-cell differentiation have led to partially contradictory results. PGE₂ has been shown to inhibit IL-4-induced IgE synthesis (19), and it was found to inhibit B-cell proliferation induced by SAC but not that induced by PWM (29). PGE₂ also inhibits production of Th1 but not Th2 lymphokines (2). In the murine model, however, PGE₂ was shown to enhance IgE and IgG1 production (17, 25). On the other hand, indomethacin inhibits PWM-induced B-cell differentiation, and PGE₂ reverses the effect of indomethacin (4). Furthermore, LTB₄ enhances proliferation and Ig production of B cells (34), but LTC₄ was reported to inhibit Ig synthesis (1).

Our data show that indomethacin and NDGA inhibit IL-4-induced IgG4 and IgE synthesis. The inhibitory effect of NDGA is consistent with the results of Yamaoka et al., who found enhancement of B-cell differentiation by LTB₄ (34). However, we were unable to restore NDGA-mediated inhibitory effect by LTB₄. Consequently, it is likely that NDGA-induced inhibition was not due to inhibition of LTB₄ only. Similarly, the indomethacin-mediated inhibitory effect did not appear to be mediated by PGE₂ only, because we were unable to restore the effect by adding exogenous PGE₂. These results suggest that other cyclooxygenase pathway products, in addition to PGE₂, play a role in the regulation of IgE synthesis as well. NDGA and indomethacin also inhibited PWM-induced production of IgM and total IgG. Consequently, AA metabolites do not specifically regulate Ig isotype switching but act on B-cell differentiation in a more general way. However, indomethacin failed to inhibit PWM-induced Ig responses in PBMCs derived from atopic dermatitis patients. This may be due to higher spontaneous release of AA metabolites from the PBMCs of atopic patients. Indeed, increased production of PGE₂ in atopic dermatitis patients has been reported (12). The mechanisms underlying indomethacin-mediated inhibition at cellular level remain to be determined. Nevertheless, cyclooxygenase products have been shown to elevate intracellular cyclic AMP levels (25); this leads to the translocation of protein kinase C to the B-cell nucleus, resulting in B-cell activation (3). Therefore, reduction in the intracellular cyclic AMP levels is one potential mechanism of indomethacin-mediated inhibitory effect.

The contradictory results obtained in the studies on the effects of AA metabolites in B-cell differentiation may be due to the different culture conditions used in these studies. Unless the experiments are performed in the presence of specific inhibitors of lipoxygenase or cyclooxygenase pathways (e.g.

NDGA and indomethacin), the cultures may already contain optimal amounts of AA metabolites, and it is not known whether the effects obtained are due to the metabolite studied or to the additive or synergistic effect of that metabolite and another metabolite present in the cultures. We demonstrate here significant suppressive effects by inhibitors of AA metabolism on IgE and IgG4 synthesis, indicating that they do indeed play an important role in the regulation of B-cell differentiation and in IgE and IgG4 synthesis. In addition, our data show that the PBMCs of healthy and atopic persons are equally sensitive to IFN- α -, IFN- γ -, and IL-2-mediated inhibition of IgE synthesis.

Acknowledgments

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