

ANDOLAST ACTS AT DIFFERENT CELLULAR LEVELS TO INHIBIT IMMUNOGLOBULIN E SYNTHESIS

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The anti-asthmatic agent andolast is thought to inhibit the release of allergic mediators, but its mechanism of action is not fully understood. We investigated whether the compound inhibits immunoglobulin E (IgE) synthesis and tested the hypothesis that andolast affects immunoglobulin class switching. Interleukin (IL)-4 and the interaction of CD40 expressed on B cells with its ligand on T cells are necessary for IgE synthesis. Thus, peripheral blood mononuclear cells (PBMCs) from 40 asthmatic, 16 non-asthmatic allergic, and 9 normal donors were stimulated with IL-4 and/or anti-CD40 antibody. T cells from 9 additional allergic donors were activated with anti-CD3/CD28 antibodies to express IL-4 mRNA. After incubation in the absence or presence of test compounds, immunoglobulin concentrations were measured by enzyme immunoassay, and mRNA levels were analyzed by RT-PCR. Andolast significantly inhibited IgE synthesis by stimulated PBMCs from both asthma patients and combined allergic/normal donors. In mechanistic studies, andolast was found to act at different cellular levels. Firstly, it reduced by about 45% ($p < 0.05$) the levels of IL-4 mRNA in T cells stimulated with anti-CD3/CD28. Secondly, andolast reduced by about 36% ($p < 0.05$) the expression of epsilon (ϵ) germline transcripts in PBMCs stimulated with IL-4/anti-CD40. Thirdly, the effect of andolast on immunoglobulin synthesis was selective in that the production of IgG4 antibodies was not significantly inhibited. Our findings, while supporting the evidence that andolast is effective for the treatment of asthma, provide new insights into its mechanism of action.

Andolast is an N-phenylbenzamide derivative under clinical development for the treatment of asthma. *In vitro* and *in vivo* experimental studies have demonstrated that the compound has anti-allergic, anti-inflammatory, and bronchoprotective properties. For instance, andolast protects guinea pigs against antigen-induced bronchial anaphylaxis, being more potent than disodium cromoglycate

(DSCG) in delaying the onset of bronchoconstriction (1-2). Clinical studies in patients with asthma have shown that andolast prevents the airway hyperresponsiveness to different challenges, with favorable pharmacokinetics and a good safety profile (3-5).

Asthma is a chronic inflammatory disease of the airways in which many cells and cellular

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elements play a role. In the hunt for genes and molecules associated with asthma pathogenesis, recent research has focused on several major areas, including the production of allergen-specific immunoglobulin E (IgE) antibodies (6). It is now clear that two signals are necessary for IgE synthesis by human B lymphocytes. The first one is provided by interleukin (IL)-4 or IL-13 and results in epsilon (ϵ) germline transcription, an early event in isotype switching to IgE. The second signal, delivered by the interaction of CD40 expressed on B cells with its ligand (CD40L) on activated T cells, promotes genomic DNA rearrangement, the expression of mature IgE transcripts, and IgE protein synthesis (7–10). Consistent with this, antibodies against CD40 can replace T-cell-derived signals *in vitro*, thereby triggering IgE production by B cells (11). Glucocorticoids, which up-regulate the expression of CD40 ligand (12) and whose levels correlate with serum IgE concentrations in allergic subjects (13), may be used as an alternative.

The exact mechanism whereby andolast exerts its bronchoprotective activity is not fully understood. Because the compound is mostly active in those experimental models involving IgE-mediated effects, there is reason to believe that it may function by inhibiting the release of allergic mediators (2).

The purpose of this study is to evaluate the effects of andolast on the events required for antibody isotype switching to IgE. Peripheral blood mononuclear cells (PBMCs) from atopic and non-atopic asthmatic subjects were incubated with andolast to test its ability to inhibit IgE synthesis. Moreover, peripheral blood cell preparations from non-asthmatic allergic and normal subjects were used to gain more insight into the mechanism of action of the compound.

MATERIALS AND METHODS

This study was carried out in accordance with the principles of the Helsinki Declaration of 1975, as amended in 2000 (14).

Materials

Andolast was synthesized at Rottapharm (Monza, Italy), whereas disodium cromoglycate (DSCG) and beclomethasone were purchased from Sigma (St. Louis, MO). Stock solutions (10^{-3} M) were prepared in sterile distilled water except for beclomethasone, which was

dissolved in 100% dimethyl sulfoxide (DMSO).

RPMI 1640 + GlutaMAX I and Dulbecco's phosphate-buffered saline (PBS) were purchased from Invitrogen (Carlsbad, CA). Fetal calf serum (FCS), gentamicin, cycloheximide, Trypan blue, and Histopaque-1077 were obtained from Sigma (St. Louis, MO). Recombinant anti-human CD3 ϵ (clone UCHT1), anti-human CD28 (clone 37407) and anti-human CD40 (clone 82111) antibodies, and IL-4, were purchased from R&D Systems (Minneapolis, MN). A different anti-human CD40 clone (626.1), kindly provided by Dr D. Vercelli (15), was used in studies with PBMCs from non-asthmatic allergic and normal donors. Alamar Blue was purchased from AbD Serotec (Martinsried/Planegg, Germany).

Studies with PBMCs from patients with asthma

Asthmatic subjects (22 atopic and 18 non-atopic) were recruited from outpatients at the Respiratory Medicine Unit of the Department of Internal Medicine (University of Brescia, Italy). The diagnosis of asthma was made by clinical history (dry cough, wheezing, and shortness of breath), pulmonary function parameters, and presence of airway hyperresponsiveness to methacholine. Atopy was defined as the presence of skin reactivity and assessed by skin prick tests (positive response to the major seasonal aeroallergens of our country, house-dust mites and moulds). Venous blood was drawn after informed consent was obtained. Purification of PBMCs was performed at room temperature using a commercially available kit from Miltenyi Biotec (Auburn, CA), according to the manufacturer's instructions. Briefly, 10 ml of heparinized blood were diluted in PBS-ethylenediaminetetraacetic acid (EDTA; 2 mM) to a final volume of 35 ml. These were carefully laid over 15 ml of Histopaque-1077 in a conical tube and centrifuged at 400 $\times$ g for 30 minutes at 20°C. The upper layer was aspirated leaving the mononuclear cell layer undisturbed at the interphase. The interphase cells (PBMCs) were transferred to a new conical tube filled with PBS-EDTA and centrifuged at 300 $\times$ g for 10 minutes at 20°C. For removal of platelets, the cell pellet was resuspended in PBS-EDTA and centrifuged at 200 $\times$ g for 15 minutes at 20°C. The supernatant was removed and the washing step was repeated. Cells were resuspended in 0.5 ml RPMI 1640-GlutaMAX I medium supplemented with 10% FCS and gentamicin (50 μ g/ml) and were counted with Trypan blue to evaluate their vitality.

PBMCs (1.5×10^6 cells/ml) were cultured in suspension for 12 days at 37°C in a humidified atmosphere of 5% CO₂ in air, in the absence or presence of IL-4 (5 ng/ml) and anti-CD40 monoclonal antibody (mAb; 1 μ g/ml). Test drugs or their vehicles were added together with the stimuli. Control cultures for the evaluation of preformed immunoglobulin were set up in the presence

of cycloheximide (10 µg/ml). After 12 days of culture, supernatants were assessed for total IgE by Enzyme-Linked ImmunoSorbent Assay (ELISA; Dasit, Cornaredo, Italy).

Because our previous studies had shown marked inter-donor variability in the IgE response to IL-4 and anti-CD40 mAb, only subjects whose PBMCs produced at least 5 ng/ml IgE upon stimulation (in the absence of test drugs) were eligible for inclusion in the current analysis. Responders were those patients whose PBMCs responded to andolast with any decrease in IgE levels compared to their respective controls (i.e., cells stimulated with IL-4/anti-CD40).

Mechanistic studies with blood cell preparations from allergic/normal donors

For this part of the study, blood samples were collected from a total of 34 donors (25 allergic and 9 normal). Allergic donors were non-asthmatic subjects with self-reported history of a positive skin prick test to at least one common allergen. Some allergic subjects were tested more than once, but not more than twice for each set of experiments.

■ *Studies with PBMCs from non-asthmatic allergic and normal donors*

PBMCs were isolated as above described. IL-4- and anti-CD40 mAb-stimulated PBMCs were cultured in the absence or presence of andolast (0.1-1 µM). After 12 days, supernatants were harvested and assayed for IgE and IgG4 levels by ELISA (Dakit, Cornaredo, Italy).

■ *IL-4, IL-5, and IL-13 mRNA expression in Th2 lymphocytes*

CD4⁺-chemoattractant receptor-homologous molecule (CRTH2)⁺ lymphocytes, which are thought to represent a subpopulation of Th2-differentiated cells (16), were obtained by magnetic cell sorting (MACS System, Miltenyi Biotec). We started from 30 ml of peripheral blood from non-asthmatic allergic donors and followed the manufacturer's instructions. The preparation can be subdivided into three steps: 1- PBMCs purification; 2- depletion of non-CD4⁺ cells; 3- positive selection of CD4⁺-CRTH2⁺ Th2 cells. CD4⁺CRTH2⁺ lymphocytes were counted with Trypan blue, and purity (>90%) was determined by FACS analysis. Cells were resuspended in RPMI 1640 + GlutaMAX I + gentamicin (50 µg/ml) + 10% FCS and plated in 24-well plates in a final volume of 400 µl/well. Cells were grown in suspension at 37°C in a 5% CO₂ atmosphere. CD4⁺CRTH2⁺ lymphocytes (20.000-40.000 cells/well) were plated in anti-CD3ε (6 µg/ml) pretreated plates (i.e., incubated for 2.5 h at 37°C and thereafter conserved at 4°C). Just before use, the unbound antibody was removed by aspiration and wells were washed twice with PBS. CD4⁺CRTH2⁺ cells were

incubated for 24 h under the following conditions: 1- control (without any stimulation); 2- anti-CD3ε (6 µg/ml) + anti-CD28 (anti-CD3/CD28) (3 µg/ml); 3- anti-CD3/CD28 + andolast (10 µM).

Total RNA was purified using the ABI Prism™ 6100 Nucleic Acid PrepStation, an RNA isolation platform from Applied Biosystems (Foster City, CA), and stored at -80°C.

Total RNA was retrotranscribed with the High-Capacity cDNA Archive Kit (Applied Biosystems), adding 50 µl of total RNA to 50 µl of reaction mix.

Transcript levels were measured by relative quantitative Real-Time polymerase chain reaction (Real-Time PCR). Probes and primers were purchased from Applied Biosystems as TaqMan® Gene Expression Assays, except for the endogenous control GAPDH (glyceraldehyde-3-phosphate dehydrogenase), which was provided as PDARs (TaqMan® Pre-Developed Assay Reagents). The reaction was performed using the ABI Prism™ 7000 Sequence Detection System, and data were normalized to GAPDH levels with the aid of the Relative Quantification/RQ Software (Applied Biosystems). Each sample was analyzed in triplicate.

■ *Semi-quantitative RT-PCR analysis of ε germline transcripts*

In selected experiments PBMCs were harvested 3 days after stimulation, and total RNA was extracted using the Totally RNA kit from Ambion (Austin, TX) according to the manufacturer's instructions. Subsequently, cDNA was prepared using SuperScript II RT (200U; Gibco BRL, Grand Island, NY). Amplification of ε germline and control GAPDH transcripts was performed as previously described (17), except that amplification products were resolved on a 1.5% agarose gel and visualized under UV light following ethidium bromide staining. The number of amplification cycles was such that the maximum signal intensity for a set of samples was within the linear portion of a product vs. template amplification curve. Amplicons of the expected size (409 bp for ε germline and 438 bp for GAPDH) were quantitated by scanning densitometry, using the Molecular Analyst software (BioRad, Hercules, CA). Results were normalized to the intensity of GAPDH bands. The same procedure was used in experiments assessing γ4 germline mRNA, as previously reported (18).

Statistics

Data were analyzed by ANOVA for repeated measures, followed by post-hoc all pairwise multiple comparison procedures (Holm-Sidak method). When the assumption of normality or equality of variance was not met, the Friedman repeated measures analysis of variance on ranks was performed, followed by Dunn's method or

Student-Newman-Keuls method as all pairwise multiple comparison post-hoc test.

When comparing two groups, paired t-test was used or, when the assumption of normality or equality of variance was not met, the Wilcoxon Signed Rank Test was applied. Unless otherwise indicated, data are given as mean $\pm$ SEM; $p < 0.05$ was considered significant.

RESULTS

Andolast inhibits IgE synthesis by stimulated PBMCs from asthma patients

We first evaluated the potential for andolast to inhibit IgE synthesis in a target population. PBMCs from 40 patients with asthma (22 atopic and 18 non-atopic) were stimulated with recombinant IL-4 and anti-CD40 mAb in the presence or absence of test compounds. After 12 days of incubation, IgE levels in the supernatants of stimulated cells were, on average, much higher than those of unstimulated cells (13.9 ± 2.0 vs. 1.8 ± 0.8 ng/ml; $n=40$). However, PBMCs from 13 patients were excluded from the analysis because of poor response to the stimulus (IgE levels <5 ng/ml), as specified in the protocol. One additional subject was excluded due to high IgE levels in the supernatant of unstimulated cells

(30.0 ng/ml), which did not increase further upon stimulation (30.8 ng/ml).

Thus, PBMCs from 26 patients (15 atopic and 11 non-atopic) were included in the final analysis. Treatment of these PBMCs with IL-4/anti-CD40 caused a nearly 15-fold increase in IgE levels, and andolast ($10 \mu\text{M}$) significantly inhibited this response (Fig. 1A). In line with published data, the glucocorticoid beclomethasone enhanced ($p=0.001$), whereas DSCG reduced (although not significantly) IgE synthesis in response to IL-4/anti-CD40 (12, 19). None of the compounds affected cell viability, as determined by Alamar blue assay (not shown).

Andolast was active in cells from both atopic and non-atopic patients (Fig. 1B), but statistical analysis did not show significant differences, most likely because of the small number of patients in each of the two subsets. When only responders (i.e., patients whose PBMCs responded to andolast with a decrease in IgE levels; 18 out of 26) were included in the analysis, the inhibition of IgE synthesis reached 46.6% (11.1 ± 1.9 vs. 20.9 ± 3.2 ng/ml, $p<0.01$; Fig. 1B).

We focused on a single concentration of andolast as preliminary experiments had indicated that concentrations lower than $10 \mu\text{M}$ did not affect IgE

Table I. IgE synthesis by PBMCs from allergic and normal donors: effect of andolast.

Treatment	IgE, ng/ml		
	Allergic donors	Normal donors	Overall
Control	1.2 ± 0.5 ($n=16$)	0.3 ± 0.05 ($n=9$)	0.9 ± 0.3
IL-4	26.5 ± 5.9 ($n=13$)	12.3 ± 6.4 ($n=7$)	21.5 ± 4.6
IL-4 + andolast $0.1 \mu\text{M}$	34.4 ± 8.8 ($n=13$)	11.5 ± 6.5 ($n=7$)	26.4 ± 6.5
IL-4 + andolast $1 \mu\text{M}$	$10.0 \pm 3.4^*$ ($n=13$)	5.0 ± 3.4 ($n=7$)	$8.2 \pm 2.5^\dagger$
Anti-CD40	0.9 ± 0.3 ($n=16$)	0.5 ± 0.1 ($n=6$)	0.82 ± 0.2
IL-4 + anti-CD40	112.7 ± 28.4 ($n=16$)	20.0 ± 3.1 ($n=9$)	79.3 ± 20.2
IL-4/anti-CD40 + andolast $0.1 \mu\text{M}$	95.1 ± 24.0 ($n=16$)	15.8 ± 3.4 ($n=9$)	66.5 ± 17.1
IL-4/anti-CD40 + andolast $1 \mu\text{M}$	$54.6 \pm 14.5^\ddagger$ ($n=16$)	17.3 ± 2.4 ($n=9$)	$41.2 \pm 9.9^*$

Values are expressed as mean $\pm$ SEM. * $p<0.05$, Dunn's method; $^\dagger p<0.01$, Dunn's method; and $^\ddagger p<0.001$, Holm-Sidak method vs. respective stimulus in the absence of andolast

Table II. IgG4 synthesis by PBMCs from allergic and normal donors: effect of andolast.

Treatment	IgG4, ng/ml		
	Allergic donors	Normal donors	Overall
Control	2.4±1.2 (n=15)	2.9±0.9 (n=8)	2.5±0.9
IL-4/anti-CD40	14.6±3.8 (n=15)	8.8±2.2 (n=8)	12.6±2.6
IL-4/anti-CD40 + andolast 0.1 µM	16.2±3.9 (n=15)	7.9±1.2 (n=8)	13.3±2.7
IL-4/anti-CD40 + andolast 1 µM	10.8±2.5 (n=15)	7.6±1.5 (n=8)	9.7±1.7

Values are expressed as mean ±SEM

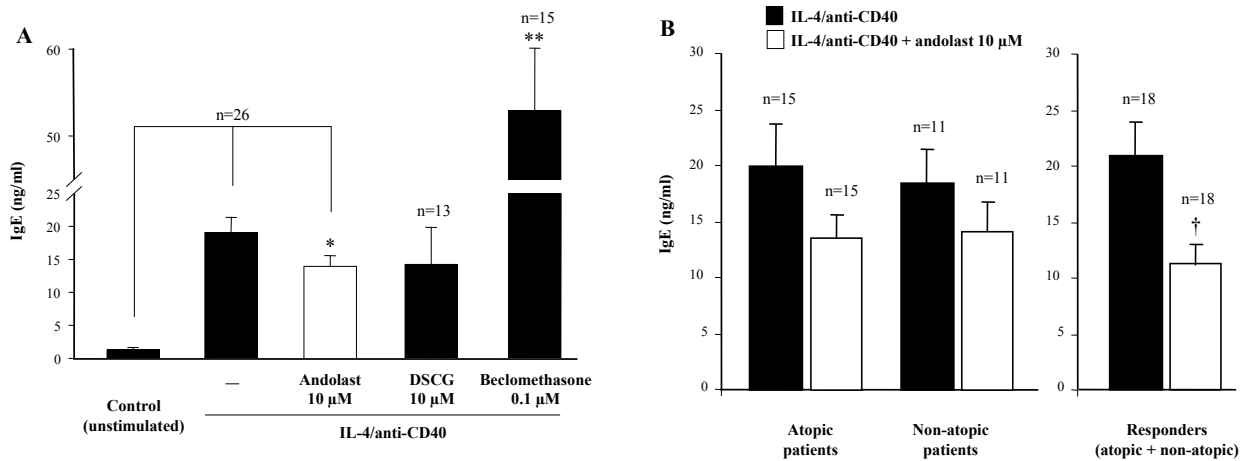


Fig. 1. IgE synthesis by PBMCs from asthmatic patients: effects of andolast, disodium cromoglycate (DSCG), and beclomethasone. Cells were stimulated with IL-4/anti-CD40 in the absence or presence of test compounds. PBMCs were cultured for 12 days, then supernatants were harvested and IgE levels were measured by ELISA. Values are expressed as mean±SEM. (A) aggregate analysis; (B) subgroup analysis. * $p < 0.05$, † $p < 0.01$, and ** $p = 0.001$ vs. IL-4/anti-CD40-treated cells, Wilcoxon Signed Rank Test.

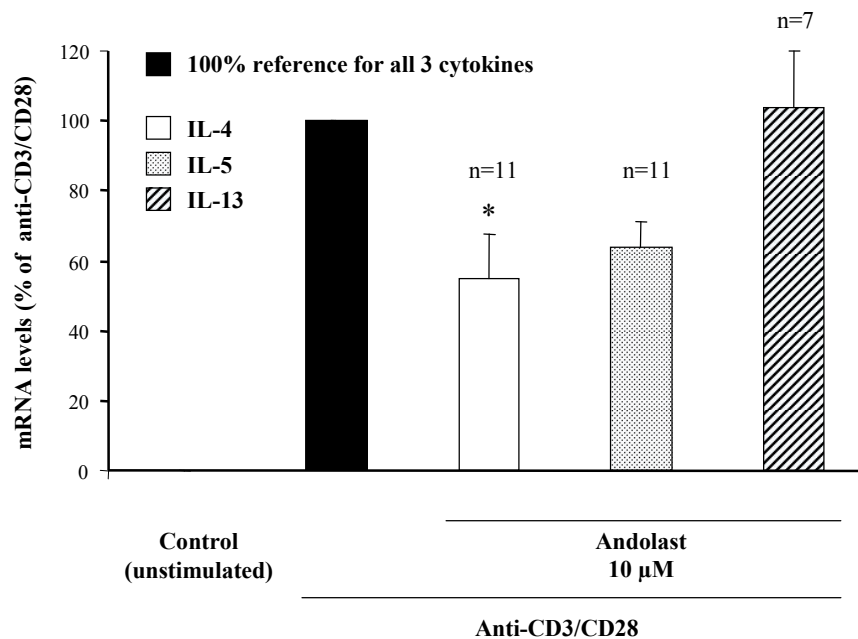


Fig. 2. Effect of andolast on the levels of IL-4, IL-5, and IL-13 mRNA. Th2 cells were stimulated for 24 h with anti-CD3/CD28 in the absence or presence of andolast (10 µM). Transcript levels were measured by Real-Time PCR. Data were normalized to GAPDH levels, and each sample was analyzed in triplicate. * $p < 0.05$ vs. anti-CD3/CD28-treated cells, *t*-test.

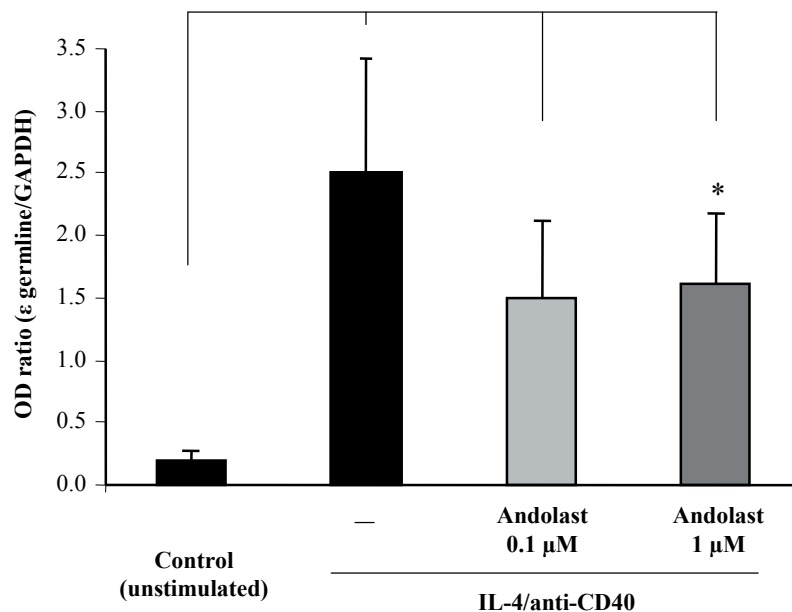


Fig. 3 Effect of andolast on the expression of ε germline transcripts. A semi quantitative RT-PCR method was used to assess mRNA levels in PBMCs from allergic and normal donors. Results are expressed as the ratio between the intensity of the ε germline and GAPDH bands, as measured by densitometry analysis. * $p < 0.05$ vs. IL-4/anti-CD40-treated cells, Student-Newman-Keuls method.

synthesis (not shown).

Mechanistic studies

- *Andolast reduces the levels of IL-4 mRNA in Th2 lymphocytes stimulated with anti-CD3/CD28*

The expression of Th2-derived cytokines is essential for IgE synthesis. To evaluate whether andolast can interfere with this event, we used Th2 lymphocytes isolated from the peripheral blood of allergic individuals (n=9). Stimulation of these cells with anti-CD3/CD28 antibodies prompted a marked increase in IL-4, IL-5, and IL-13 transcript levels. When Th2 lymphocytes were stimulated in the presence of andolast (10 μ M), the mean levels of IL-4 mRNA were reduced by about 45% ($p < 0.05$ vs. anti-CD3/CD28 alone; Fig. 2). IL-5 mRNA was reduced by 36%, but the effect was not significant. Conversely, andolast did not affect IL-13 transcript accumulation in response to anti-CD3/CD28.

- *Andolast inhibits IgE synthesis by stimulated PBMCs from non-asthmatic allergic donors*

IgE production by unstimulated PBMCs was greater in cells from allergic subjects than in cells from normal donors. IL-4, but not anti-CD40, induced IgE synthesis by PBMCs from allergic and normal donors, and the combination of IL-4 and anti-CD40 crosslinking yielded a synergistic effect. Overall, andolast (1 μ M) significantly inhibited IgE synthesis regardless of whether it was induced by IL-4 alone or in combination with anti-CD40 (Table I). Notably, andolast inhibited IgE synthesis by up to 61.9% in cells stimulated with IL-4 alone. Subgroup analysis showed significant inhibition in PBMCs from allergic but not normal individuals (Table I).

- *Andolast reduces the expression of ϵ germline transcripts*

In an attempt to further characterize the molecular mechanisms whereby andolast inhibits IgE synthesis, we tested the hypothesis that the compound affects ϵ germline transcription. Consistent with the results obtained when evaluating IgE levels, IL-4 in combination with anti-CD40 increased the expression of ϵ germline transcripts in PBMCs cultures (14-fold over control values). Andolast (1 μ M) reduced this response by about 36% ($p < 0.05$; Fig. 3), thus suggesting an effect on the early steps of B-cell commitment to IgE switching. When $\gamma 4$ germline mRNA was analyzed in a pilot experiment,

no inhibitory activity was found (data not shown).

- *Andolast does not affect IgG4 synthesis*

IL-4 induces IgG4, as well as IgE, synthesis, and specific IgG4 responses are thought to protect against IgE-dependent reactions (18, 20). Because our preliminary experiments had suggested that $\gamma 4$ germline transcription was not inhibited by andolast, we tested whether this could translate into unaffected IgG4 levels. Indeed, andolast did not significantly inhibit IgG4 synthesis by PBMCs stimulated with IL-4/anti-CD40 (Table II), thus pointing to a selective effect on IgE.

DISCUSSION

We report that andolast selectively inhibits IgE synthesis by human PBMCs. In addition, we provide a possible explanation for how the compound affects the molecular events that regulate isotype switching to IgE.

In experiments with cells from asthma patients, the response to andolast followed a bimodal distribution - that is, PBMCs from about one third of the patients included in the analysis (8 out of 26) did not respond to andolast. Other authors have reported a similar pattern of response for nedocromil sodium and disodium cromoglycate (11, 19). However, our aim is not to compare the efficacy of these compounds. Disodium cromoglycate was used as an active control because it is known to inhibit IgE synthesis by stimulated B lymphocytes (19).

Our main goal is to evaluate the potential for andolast to reduce the humoral response in asthma. Even before omalizumab was approved for the treatment of allergic asthma, it was clear that the inhibition of IgE-mediated effects results in decreased airway inflammation, eosinophilia, and frequency of asthma exacerbations. The important clinical outcomes associated with anti-IgE therapy must be acknowledged, but omalizumab has modest effects on symptoms such as airway hyper-responsiveness and airflow obstruction (21-22). Differently from omalizumab, andolast effectively counteracts bronchoconstriction and modulates IgE-mediated responses without directly targeting circulating immunoglobulins (3-4). The links between inflammation and airway function in asthma are not clear-cut (22), so it is reasonable

that drugs targeting upstream regulators of allergic inflammation may have broader activity.

Previous reports have emphasized that andolast is an active anti-allergic agent (1-2), but its mechanism of action is not fully understood. In our mechanistic studies, andolast was found to act at different cellular and molecular levels. Firstly, it reduced IL-4 mRNA in activated T cells from allergic donors. IL-4 enhances the antigen-presenting capacity of B cells, promotes Th2 responses, and - most importantly - it is the main inducer of immunoglobulin class switching to IgE (8, 23-24). The fact that IL-13 transcript levels were not affected by andolast argues in favor of a selective activity, especially if one considers that IL-13 is more widely produced than IL-4 but less potent in inducing IgE isotype switch (8, 23, 25). Despite the lack of statistical significance, due consideration should also be given to the inhibitory effect of andolast on IL-5, the most important cytokine involved in eosinophil chemotaxis (23). Because we believe in the clinical relevance of this finding, a study in patients with asthma was specifically designed and is currently under way to address the issue.

Before continuing with mechanistic studies, we demonstrated that andolast also inhibited IgE synthesis by PBMCs from allergic/normal donors. Subgroup analysis showed significant inhibition only in cells from allergic individuals, but we cannot rule out that a significant effect in cultures from normal donors was masked by the limited sample size. Studies with other agents corroborate this hypothesis. Resiquimod, an immune modulator, appears to be non-selective between allergic and normal donors (26). Loh et al. have reported a strong inhibition of IgE synthesis by nedocromil sodium in PBMCs from healthy individuals, although their results are focused on responders (11).

Further mechanistic studies showed that andolast reduced the expression of ϵ germline mRNA, suggesting that it may inhibit ϵ germline transcription, the initial step of B-cell activation and isotype switching to IgE. This mechanism differs from those of both disodium cromoglycate and nedocromil sodium, which act at the level of deletional switch recombination. The difference is relevant because there are common and isotype-specific mechanisms involved in immunoglobulin class switching, and

CD40-mediated switch recombination is supposed to be a common mechanism. Accordingly, nedocromil sodium and disodium cromoglycate inhibit IgG4 synthesis even though they do not affect $\gamma 4$ germline transcription (11, 19).

Andolast did not inhibit IgG4 synthesis to a significant degree, thus supporting the specificity of its mechanism of action at the transcriptional level. A sparing effect on IgG4 could be of clinical relevance in asthma, given that allergen-specific IgG4 antibodies competitively block the events mediated by IgE (e.g., histamine release by basophils) (27-28). Recent findings suggest that a protective IgG4 response is associated with the improvements observed in rhinitis patients after immunotherapy (29). Interestingly, while safer regimens of immunotherapy are now available for patients with IgE-mediated rhinitis and/or asthma (30), the combination of anti-IgE treatment with immunotherapy results in enhanced efficacy (20).

Until recently, isotype switching to IgE has been considered an event restricted to lymphoid tissues, with IgE-positive cells migrating from these centers to peripheral sites of allergic inflammation. If so, the inhibitory activity of andolast on ϵ germline transcription would be useless, because the compound is formulated for inhalation and is expected to have minimal systemic effects (5). However, a recent study of explanted tissues from the airway mucosa (thus devoid of cell recruitment) has revealed that isotype switching to IgE also occurs at the peripheral level (31). This finding validates the use of inhaled andolast for the treatment of asthma and enhances the relevance of our results.

At the same time we acknowledge that the present study has some limitations. Firstly, we did not demonstrate a concentration-dependent effect of andolast in cells from asthma patients. Because preliminary results had indicated that andolast did not affect IgE synthesis at concentrations lower than 10 μ M, we focused on this reasonably low concentration to minimize the amount of blood drawn from these patients. Secondly, the compound was 10 times more potent in cells from allergic/normal individuals than in those from asthma patients. There is no clear reason to account for this difference, especially between allergic and asthmatic individuals. One explanation may be that the anti-

CD40 antibody used was not the same throughout the study.

Even so, our results demonstrate that andolast acts at different cellular levels to inhibit IgE synthesis, supporting the evidence that it is effective for the treatment of asthma. Studies are under way to evaluate whether andolast also inhibits IgE synthesis *in vivo*.

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