

Effect of Topical Fluticasone Propionate on the Mucosal Allergic Response Induced by Ragweed Allergen and Diesel Exhaust Particle Challenge

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Glucocorticoids block the local allergic response in a variety of ways. However, studies have also shown that glucocorticoids increase *in vitro* IgE synthesis and that treatment with corticosteroids may result in elevated serum IgE concentrations. The ability of topical glucocorticoids to modulate the mucosal IgE response has not been elucidated. We studied the effect of topical steroid (fluticasone propionate) treatment on the local allergic antibody response induced by challenge with either allergen or diesel exhaust particles (DEP). A parallel group study was performed with ragweed-allergic subjects, each subject serving as his/her own control. Nasal provocation challenges were performed on three groups. One group received ragweed allergen, another diesel exhaust particles, and the third saline. The study was repeated following 1 week of treatment with intranasal fluticasone propionate. Each group received the same challenge as before. The concentrations of total immunoglobulins (IgE, IgG, IgA, and IgM), anti-ragweed antibody, IgE- and IgA-secreting cells, epsilon (ϵ) mRNA, and cytokine mRNAs (IL-2, -4, -5, -6, TNF- α , INF- γ) were measured in nasal lavages performed before and at various time points after challenge. Treatment with fluticasone propionate for 7 days caused a decrease in the concentrations of nasal IgE protein, IgE-producing cells, total ϵ mRNA, and all the cytokine mRNAs tested. Furthermore, treatment with fluticasone propionate inhibited the production of allergen-specific IgE and cytokine mRNAs following challenge with ragweed antigen. However, fluticasone treatment did not significantly inhibit the enhancement of mucosal IgE production or cytokine mRNAs observed following nasal challenge with DEP. These results indicate that 1-week treatment with topical fluticasone propionate was effective in blocking local effects of allergen exposure but was unable to inhibit the adjuvant-like effect of DEP. © 1999 Academic Press

Key Words: IgE, human; topical corticosteroids; mucosal allergic response; allergen challenge; diesel exhaust particulate effects.

INTRODUCTION

IgE has a central role in the pathogenesis of allergic airway disease. A heightened local IgE response can be induced experimentally in allergic subjects by challenge with a relevant allergen or in nonallergic and allergic subjects alike upon challenge with certain pollutants, such as diesel exhaust particles (1). The production of IgE is under the regulation of a variety of cell contact and soluble signals provided by cytokines. Actual DNA switch recombination (i.e., isotype switching) is also dependent on a cell contact-mediated signal, which is thought to be provided by CD40–CD40L or CD2–CD58 interactions *in vivo* (2, 3). Such contact signals can be replaced *in vitro* by corticosteroids such as hydrocortisone (4). Furthermore, hydrocortisone selectively stimulates isotype-switched sIgE⁺ and sIgG4⁺ B cells from atopic subjects and results in enhanced spontaneous IgE and IgG4 production (5).

In this study we investigated the effects of a topical steroid, fluticasone propionate (Flonase), on the local allergic inflammatory response. We show that in contrast to the previously reported increase in serum IgE following glucocorticoid treatment (6), treatment with fluticasone propionate decreased the concentrations of local, nasal IgE protein and cytokine mRNA. Additionally, we show that fluticasone treatment inhibited many local immune responses, such as production of allergen-specific IgE and cytokines induced by allergen challenge, but fluticasone treatment was not able to not block local mucosal changes induced by intranasal diesel exhaust particles (DEP)¹ challenge.

MATERIALS AND METHODS

Subjects

Fifteen healthy, nonsmoking volunteers (eight men and seven women) ranging from 18 to 50 years old were

¹ Abbreviations used: DEP, diesel exhaust particles; D.U., densitometry units; PCR, polymerase chain reaction.

recruited for this study. Subjects had a history of allergic upper airway disease (allergic rhinitis) and a positive skin prick test (wheal ≥ 4 mm with surrounding erythema) to Eastern (short) ragweed. All had lived in the greater Los Angeles area for at least 1 year. None of the volunteers had previously had any known unusual or extraordinary exposure to air pollutants. Throughout the period of the study there were no pollution alerts in the Los Angeles area. None of the subjects had used topical or systemic steroids in the 3 months prior to the study or oral antihistamines for the prior week. None had ever been on allergy immunotherapy. All studies were approved by the Human Subject Protection Committee of the University of California at Los Angeles. Treatment with fluticasone propionate (Flonase, GlaxoWellcome, Research Triangle Park, NC) consisted of the recommended dose of two sprays of 50 μ g each per nostril once daily for a period of 7 days prior to challenge, including the day of challenge.

Nasal Lavage and Ragweed or DEP Provocation Challenges

Nasal washes and provocation challenges were performed as previously described (1). Briefly, for nasal lavage, 5 ml of normal saline was delivered into each nostril of the subjects, and after 10 s the wash fluid was collected. The initial wash was discarded. The subjects then performed four subsequent nasal washes at 10-min intervals. Washes performed at the same time-point were pooled. The tubes were centrifuged at 350g for 10 min at 4°C, and the aqueous supernatants were separated from the cell pellets. When cells were needed for filter spot-ELISA or for RNA extraction, the number of nasal washes was increased to eight.

In this parallel group study each subject served as his/her own control. The subjects were separated into one of three groups with five subjects per group. Ten minutes following the last wash, one group was challenged with 200 μ l saline, another with 0.3 mg of DEP in 200 μ l saline, and the third group with ragweed allergen. In subjects receiving ragweed allergen, the nose was sprayed with 10-fold increasing doses of an extract of short ragweed containing a known amount of the antigen *Amb a* I (Hollister Stier/Baxter, Irwindale, CA). The starting dose was 10 AU, and this was increased until symptoms (sneezing/irritation) were apparent. No subject received more than 10,000 AU or less than 100 AU. Nasal lavages were performed, as above, on subjects 18 h after challenge (day 1) and on days 4 and 8. After a period of at least 4 months, the same individuals were treated for 1 week with fluticasone, after which treatment was discontinued. The subjects were then separated into the same groups as before. Each group was lavaged and received the same

challenge as before. We have previously shown that if there is a gap of 60 days or more between allergen or DEP challenges there is no priming or accumulative effect (1, 7).

DEP were obtained from light duty diesel passenger cars and processed as previously described (1).

Immunoglobulin and Albumin Determination

The concentrations of IgE, IgG, and IgA in the nasal lavage fluids were measured by isotype-specific ELISAs as previously described (1, 8). Albumin concentrations were determined in an analogous ELISA procedure (1). Ragweed-specific IgE in lavage fluid was determined using the same procedure as for total Ig isotypes except that an amplification system previously described was used with minor modifications (7, 8). The plates were coated with 10 μ g/ml *Amb a* I (Hollister Stier/Baxter, Irwindale, CA) and alkaline phosphatase-labeled anti-IgE, anti-IgG, or anti IgG4 (Tago, Burlingame, CA) at 1/3000 were used for detection. For all ELISA strategies, samples were run in duplicate, and optical density values that differed from the mean by greater than 10% were reassayed.

Measurement of IgE- and IgA-Secreting Cells

IgE- and IgA-secreting B cells were enumerated using a filter spot-ELISA as previously reported (1, 9, 10). The sensitivity of the IgE spot assay was determined by using serial dilutions of 2C4/F3 IgE-producing cells (11); direct counts of these cells were compared with the number of spots detected and used to construct a standard curve. The assay was shown to be specific because no plaques were detected when myeloma cells producing other immunoglobulin isotypes were used (1, 9). Similarly, IgA-producing myeloma cells (GM1056) (12) were used to construct a standard curve for the IgA filter spot assay. Positive reactions were recognized as circular granulated foci with diameters greater than 0.5 mm. Samples were run in quintuplicate and serially diluted.

Reverse Transcription-Polymerase Chain Reaction (PCR) Assay for Cytokines and Epsilon (ϵ) mRNA Splice Variant Expression

Total cellular RNA from the cells recovered from nasal washes was isolated and then reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD) as previously described (1). Cytokine mRNAs were measured using the same primers and conditions as previously described (7, 13, 14). The PCR strategies were based on those reported by Yamamura

et al. (13), which allow comparison of cytokine mRNA in different samples. This PCR strategy had been demonstrated to be quantitative throughout a broad range of cDNA concentrations by the use of cytokine cDNA containing plasmids. The amounts of cDNA used for each cytokine PCR were normalized to yield equivalent PCR products of β -actin, a "housekeeping gene" constitutively expressed by cells, and a direct measure of the amount of cells from which RNA was extracted. The adjusted PCR product was then electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining. PCR results were confirmed by transfer of the electrophoresed PCR products to nylon membranes (Hybond-N membrane, Amersham Corp., Arlington Heights, IL) and hybridization with ^{32}P -radiolabeled oligonucleotide probes complementary to internal sequences of the PCR products as previously reported (1, 15). The resultant autoradiograph was digitized, and the intensity of the bands was calculated by densitometry using the Biosoft Scan Analysis computer program. The mean intensity of the bands for each cytokine (in densitometry units) was thereby calculated. Maximal expression of cytokines was observed between 18 and 24 h after challenge as has previously been reported (16, 17). Cytokine protein concentrations in the lavage fluid were too low to measure by immunoassays.

Primary RNA transcripts from the single human functional ϵ heavy chain gene can be alternatively spliced to produce at least 5 different ϵ mRNA splice variants (15, 18). We have previously developed two modified polymerase chain reaction techniques to compare the relative amounts of different isoforms of ϵ mRNA produced (1). The first PCR amplified the three ϵ mRNA isoforms CH4-M1'-M2, CH4-M2', and CH4-M2'' and the housekeeping gene β -actin. The ϵ proteins encoded by these mRNAs are membrane, grande, and tail piece, respectively (19). A second PCR was used to amplify two other ϵ mRNA splices, the CH4-S form (encoding classic secreted IgE) and the alternative-spliced CH4'-CH5 form (encoding IgE chimeric). The resultant PCR products were visualized on an 8% acrylamide gel and quantified by densitometry as previously described (1). All densitometry values for ϵ isoforms were normalized to β -actin expression. All samples were run in duplicate.

Statistical Analysis

The Statview II computer package (Abacus Concepts, Berkeley, CA) for the Macintosh was used for all analyses. Comparisons within groups were done using paired *t*-tests because normality had been established. Comparisons between groups were performed using unpaired *t*-tests. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Topical Fluticasone Propionate Inhibits Baseline Mucosal IgE Production

Prior to any challenge, immunoglobulin concentrations in the nasal washes of the 15 subjects were compared immediately before and after 1-week treatment with fluticasone (Fig. 1). There was a significant decrease in total IgE concentrations in the lavage following fluticasone treatment (mean = 0.14 ± 0.09 ng/ml compared to 0.27 ± 0.15 ng/ml in the absence of treatment, $P < 0.01$) and a similar decrease in both total IgG and IgG4 ($P < 0.01$ for both) (Fig. 1A). IgA concentrations were unchanged (data not shown). There was no difference in the baseline concentrations of ragweed-specific IgE before (day 7) compared to after (day 0) treatment, but it must be recognized that these concentrations were already low because ragweed is a relatively minor antigen in Southern California and challenges were performed outside of the ragweed season.

A filter spot-ELISA was used to determine the effect of fluticasone treatment on the number of IgE- and IgA-secreting cells present in nasal washes (Fig. 1B). There was a significant decrease in the number of IgE-secreting cells detected after regular use of fluticasone for 1 week compared to baseline values ($P < 0.05$). The mean number of IgE-secreting cells fell from 0.6 per 10^6 cells at day 7 to 0.21 per 10^6 cells at day 0. There was no significant difference in the number of IgA-secreting cells following fluticasone treatment compared to pretreatment baseline concentrations.

Topical Fluticasone Propionate Inhibits Mucosal Antigen-Specific but Not Total IgE Production in Response to Challenge

We confirmed our previous findings that challenge of subjects with ragweed in the absence of fluticasone treatment resulted in elevated concentrations of ragweed-specific IgE 4 days after challenge (12.2 ± 6.1 U/ml compared with 2.5 ± 1.9 ng/ml on day of challenge, $P < 0.01$), while there was no change in IgG, IgG4, or IgA concentrations. In contrast to studies showing that treatment with systemic glucocorticoids may induce an increase in serum IgE (6), we found that treatment with fluticasone blocked the ability of subjects to mount a local ragweed-specific IgE response to ragweed challenge. At day 4 ragweed-specific IgE levels were down to a mean of 5.5 ± 5.0 U/ml (Fig. 2). Following fluticasone treatment, there was no statistically significant increase above prechallenge concentrations in ragweed-specific IgE in response to ragweed challenge at any time point. Thus, following treatment with fluticasone, ragweed challenge was not significantly different than saline challenge.

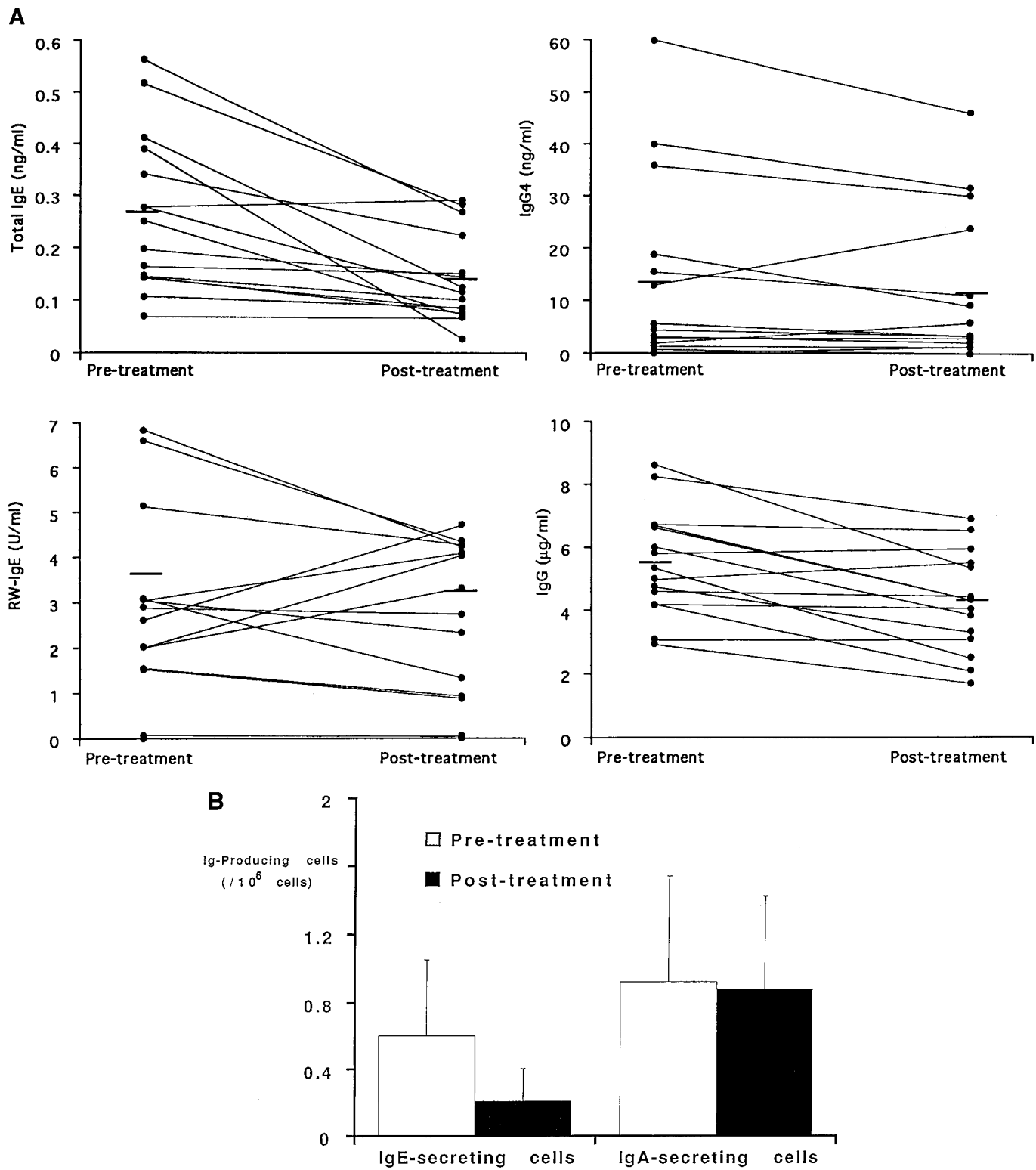


FIG. 1. Fluticasone propionate nasal spray treatment reduces the baseline concentrations of total IgE, IgG4, and IgG but not ragweed-IgE. (A) Antibody/immunoglobulin concentrations are shown from nasal lavages from 15 allergic subjects before and after treatment for 7 days with 200 μ g of fluticasone propionate. The mean is illustrated by a black bar. (B) The mean number (+1 SD) of IgE- and IgA-secreting cells found in lavages of 15 subjects before and after treatment with fluticasone are shown.

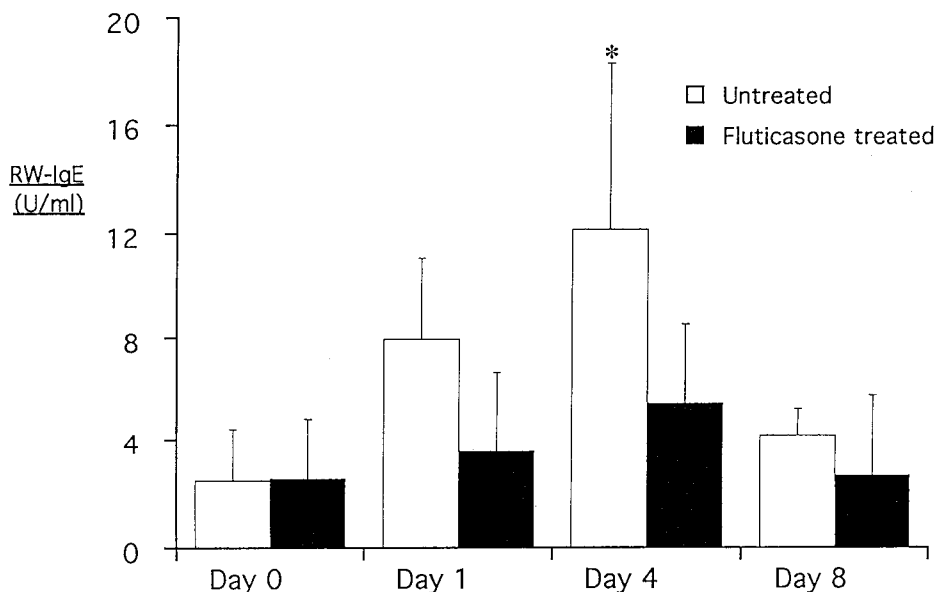


FIG. 2. The effects of fluticasone on antigen-specific IgE in ragweed-challenged subjects. Ragweed-specific IgE concentrations from nasal lavages of subjects challenged with 10,000 AU of ragweed are shown. The concentrations following identical challenge following treatment for 7 days with 200 μ g of fluticasone propionate is also shown. The mean of five subjects $\pm$ 1 SD is shown. * $P < 0.01$ vs day of challenge (day 0).

In contrast to the antigen-specific IgE results, fluticasone treatment did not interfere with the ability of subjects to respond with increased total IgE following challenge with either ragweed or DEP (Fig. 3). When subjects were treated with fluticasone and challenged with ragweed allergen, the concentrations of total IgE were significantly greater at day 4 ($P < 0.001$, mean = 2.0 ± 1.1 ng/ml) and at day 8 ($P < 0.01$, mean = 0.8 ± 0.7 ng/ml) than at day 0 (mean = 0.2 ± 0.1 ng/ml). Similar results were also observed in fluticasone-treated subjects challenged with DEP.

Failure of fluticasone propionate to block total IgE enhancement was corroborated by measurement of the number of IgE-producing cells (data not shown). Treatment with fluticasone did not alter (neither inhibiting nor augmenting) the expected increase in IgE-secreting cells observed after challenge with ragweed or DEP. The number of these cells present in washes performed 1, 4, and 7 days after challenge were higher than those observed in prechallenge washes. However, there was no significant differences in cell numbers regardless if challenges were performed prior to or after treatment with fluticasone. After challenge with saline, DEP, or ragweed, there was no increase in IgG concentrations in nasal lavage fluids over baseline concentrations at any of the time points studied regardless of whether the subjects had been treated with fluticasone or not (data not shown). Similar results were observed for IgA concentrations. No change in IgA-secreting cell numbers were observed at any time.

Topical Glucocorticoid Effects on Total ϵ mRNA and the Pattern of ϵ mRNA Splice Variants

Consistent with the total IgE protein and spot-forming cell data discussed earlier, fluticasone treatment for 7 days caused a significant decrease ($P < 0.001$) in the total concentration of ϵ mRNA in nasal lavage cells recovered at time of challenge compared with baseline. Thus the amount of ϵ mRNA present in the lavage cells following 1 week of treatment with fluticasone was only 43.2% ($\pm 10.2\%$) of that of baseline (Table 1).

We have previously shown that alternatively spliced ϵ mRNAs encode for distinctive IgE proteins. In diseases associated with high serum IgE concentrations (e.g., atopic dermatitis, parasite infestation) there is selection against one splice variant (CH4'CH5) that encodes the novel IgE protein "chimeric" (20). After 1 week of fluticasone treatment there was a significant decrease in the concentration of all of the individual ϵ mRNA splice variants compared to baseline. In the absence of any challenge, treatment with fluticasone decreased production of all isoforms equally and did not preferentially select against any one isoform ($P > 0.05$).

When the subjects were not treated with fluticasone, challenge with DEP or ragweed resulted in an increase in the total amount of ϵ mRNA and a marked selection against the production of the CH4'CH5 splice variant (1, 7). Therefore, the increase in CH4'CH5 levels following challenge with either ragweed (5.2%) or DEP

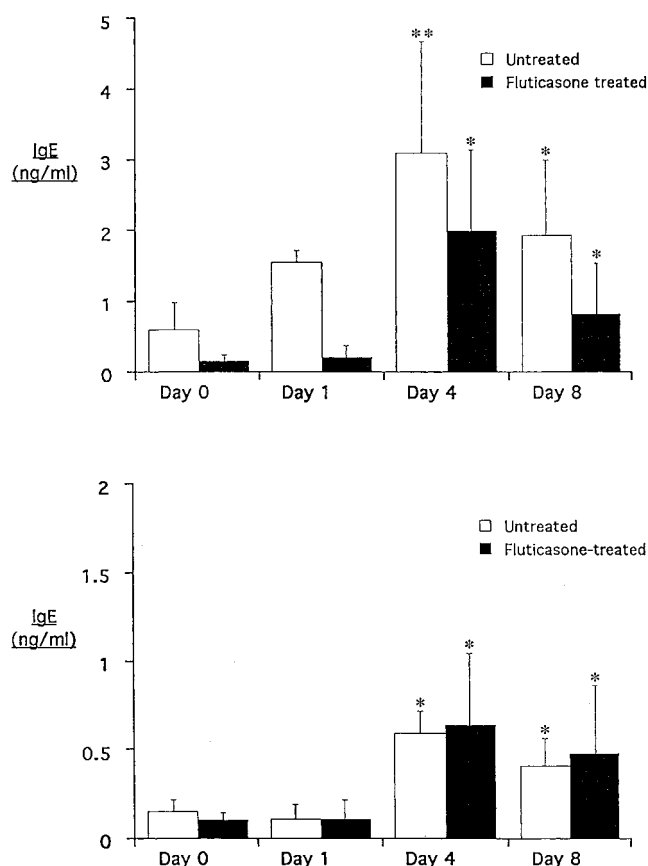


FIG. 3. The effects of fluticasone on total IgE in ragweed- and DEP-challenged subjects. Total IgE concentrations from nasal lavages of subjects challenged with 10,000 AU of ragweed (top) or with 0.3 mg DEP (bottom) are shown. The concentrations following identical challenge following treatment for 7 days with 200 μ g of fluticasone propionate is also shown. The mean of five subjects $\pm$ 1 SD is shown for each group. * P < 0.01 vs day of challenge (day 0). ** P < 0.001 vs day of challenge (day 0).

(7.5%) was significantly lower than total ϵ mRNA (29.4% and 30.25, respectively; P < 0.01). Treatment with fluticasone partially blocked the selection against production of the CH4'CH5 isoform following either ragweed (11.5%) or DEP challenge (14.0%) (Table 1) but did not block the increase in the total amount of ϵ mRNA (18.6 and 28.5% for ragweed and DEP, respectively).

Topical Glucocorticoids Effects on Cytokine mRNA Production

It has been reported that topical steroids inhibit cytokine mRNA expression in the nasal mucosa before and after allergen challenge (16). We compared the relative cytokine mRNA expression from cells recovered from nasal lavages before and after 1 week of treatment with fluticasone propionate. The results for

INF- γ and IL-6, which were the most abundant cytokines tested prior to challenge, are shown in Fig. 4. Compared to baseline concentrations (day 7), there was a reduction in the concentration of mRNA expression for INF- γ (3281 ± 1152 vs 6456 ± 1936 densitometry units (D.U.)) and IL-6 (3316 ± 794 vs 4042 ± 577 D.U.) following fluticasone treatment (day 0). A similar reduction in expression of IL-2, -4, -5, and TNF- α mRNA was also observed (data not shown).

When untreated subjects were challenged with ragweed antigen, a small but significant increase in expression for IL-4 and IL-5 was observed 18 h later (Fig. 5A). In contrast, after treatment with fluticasone and challenge with ragweed antigen, there was no increase in expression of these cytokines. Challenge with ragweed did not alter mRNA expression for any of the other cytokines tested in either untreated or fluticasone-treated subjects.

While treatment with fluticasone inhibited the ragweed-stimulated cytokine mRNA enhancement for IL-4 and IL-5, it did not block the more marked cytokine-enhanced mRNA production observed following challenge with DEP (Fig. 5B). As previously reported (7) challenge with DEP dramatically increased cytokine mRNA expression. Both untreated or fluticasone-treated subjects demonstrated increased expression of mRNA for IL-4 ($20,164 \pm 11,032$ and 8961 ± 7765 D.U.) and IL-5 ($32,157 \pm 7734$ and $25,141 \pm 9514$ D.U.) 18 h after DEP challenge. There was no difference in the relative concentrations of expression for each of the tested cytokine mRNAs in fluticasone vs untreated subjects following DEP challenge. Identical results were also seen for IL-2, -6, TNF- α , and INF- γ (data not shown).

TABLE 1

The Effects of Fluticasone on the Relative Levels of ϵ mRNA Isoforms

	Total ϵ mRNA	CH4'CH5
% Pretreatment ^a	43.2% ($\pm$ 10.3)	55.6% ($\pm$ 9.6)
Fold increase after challenge ^b		
Post-ragweed challenge—		
Untreated	29.4 ($\pm$ 6.5)	5.2 ($\pm$ 2.7)
Post-ragweed challenge—		
Fluticasone treated	18.6 ($\pm$ 5.2)	11.5 ($\pm$ 5.4)
Post-DEP challenge—		
Untreated	30.2 ($\pm$ 10.2)	7.5 ($\pm$ 4.1)
Post-DEP challenge—		
Fluticasone treated	28.5 ($\pm$ 14.2)	14.0 ($\pm$ 7.8)

^a The percentage reduction compared with baseline levels of mRNA for total and CH4'CH5 ϵ isoform from cells recovered in nasal lavages after 7 days treatment with fluticasone.

^b The relative enhancement 4 days after challenge (day 4) compared with day of challenge (day 0) for total and CH4'CH5 ϵ isoform expressed as fold increase. Levels were calculated by RT-PCR and densitometry (see Methods).

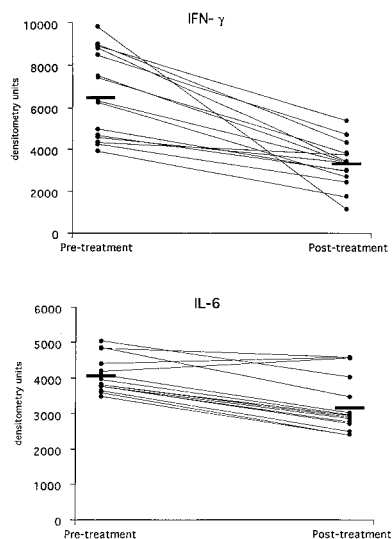


FIG. 4. Fluticasone propionate nasal spray treatment reduces the baseline concentrations of cytokine mRNA production in lavage cells. mRNA concentrations for two representative cytokines IFN- γ and IL-6 are shown from nasal lavages from 15 allergic subjects before and after treatment for 7 days with 200 μ g of fluticasone propionate. Similar results were seen in all cytokines tested. The amount of PCR product was determined by the intensity of the bands following Southern blotting measured by densitometry (see Methods). The mean is illustrated by a black bar.

DISCUSSION

Topical corticosteroids have become the cornerstone of the long-term management of chronic allergic airway inflammation in asthma and allergic rhinitis. The therapeutic benefits of oral and inhaled corticosteroids in atopic diseases are well-documented (21, 22). In this study we have demonstrated that a 1-week treatment course with fluticasone propionate, a topical glucocorticoid, caused a clear decrease in the baseline concentrations of nasal IgE protein, IgE-producing cells, ϵ mRNA, and cytokine mRNAs. Furthermore, fluticasone treatment inhibited the ability of intranasal antigen (ragweed) challenge to induce subsequent local immune changes, most notably the local production of allergen-specific IgE. However, fluticasone treatment was not able to significantly inhibit the enhanced local immune changes, such as increased IgE and increased cytokines, observed in the nose following nonspecific nasal challenge with DEP.

The ability of fluticasone to reduce baseline concentrations of IgE is important in light of studies that have raised concern about the possible enhanced *in vivo* effects of corticosteroids upon IgE production. Over 25 years ago Gunnar *et al.* reported that treatment of atopic patients with glucocorticoids resulted in elevated serum IgE concentrations (23). Since that time a variety of reports have confirmed these findings, showing that corticosteroids can enhance IgE and IgG4 syn-

thesis both *in vitro* and *in vivo* (4, 6, 24). It has also been shown that systemic glucocorticoid treatment increases both spontaneous IgE and IgG4 in B cells taken from the blood of atopic patients (5). It even has been suggested that the reliance on corticosteroids may thereby have contributed to the increased morbidity of allergic respiratory disease observed in the past 2 decades. In our study, treatment with fluticasone resulted in reduction not only in nasal IgE but also in nasal IgE-producing cells and ϵ mRNA. The difference between this and previous findings may reflect a difference in the action of glucocorticoids at the local and systemic concentration because most of the previous *in vivo* studies have examined the effect of oral steroids on systemic effects. Levels of both IgG4 and total IgG also were diminished following fluticasone treatment, suggesting that the action of the corticosteroid was not isotype-specific and limited to the IgE-regulatory pathway.

The reduction in IgE concentrations we observed is likely due to the reduction in proinflammatory cytokine concentrations, as evidenced by the decrease in cytokine mRNA levels observed after 1 week of treatment with fluticasone. It should be noted, however, that this decrease was not confined to any group of cytokines with "TH1"-, "Th2"-, and "TH0"-type cytokines being equally affected. Topical glucocorticosteroid down-regulation of cytokine expression in the upper airways demonstrated here has also been shown in the lower airways by Davies *et al.* (25). They found that the bronchial epithelium from mild asthmatics treated with topical glucocorticoids show reduced expression of a variety of cytokines. This nonspecific reduction in cytokine expression is mirrored by a reduction in ϵ mRNA expression. Some agents, such as antigen, DEP, anti-CD23, or IL-10, will preferentially select for or against certain ϵ mRNA splice variants (1, 20). In contrast, fluticasone treatment resulted in an equal decrease in the concentration of all of the individual ϵ mRNA variants. Thus, in the absence of specific antigen challenge, fluticasone blocked a variety of allergic inflammatory parameters which are probably driven by chronic antigen exposure.

Acute challenge with either allergen or DEP results in enhanced IgE responses (1, 7). While topical glucocorticoid treatment could partially or completely block some of the allergen-driven effects (specific IgE and cytokine mRNA enhancement), such treatment had minimal effects on DEP-driven responses. This is not altogether surprising because the response leading to production of IgE induced by a specific allergen (ragweed) versus that produced by the non-specific adjuvant DEP differ in several important ways (7). Intranasal challenge with DEP results in a significant increase in a broad array of cytokines (14), while challenge with allergen, in this case ragweed, results

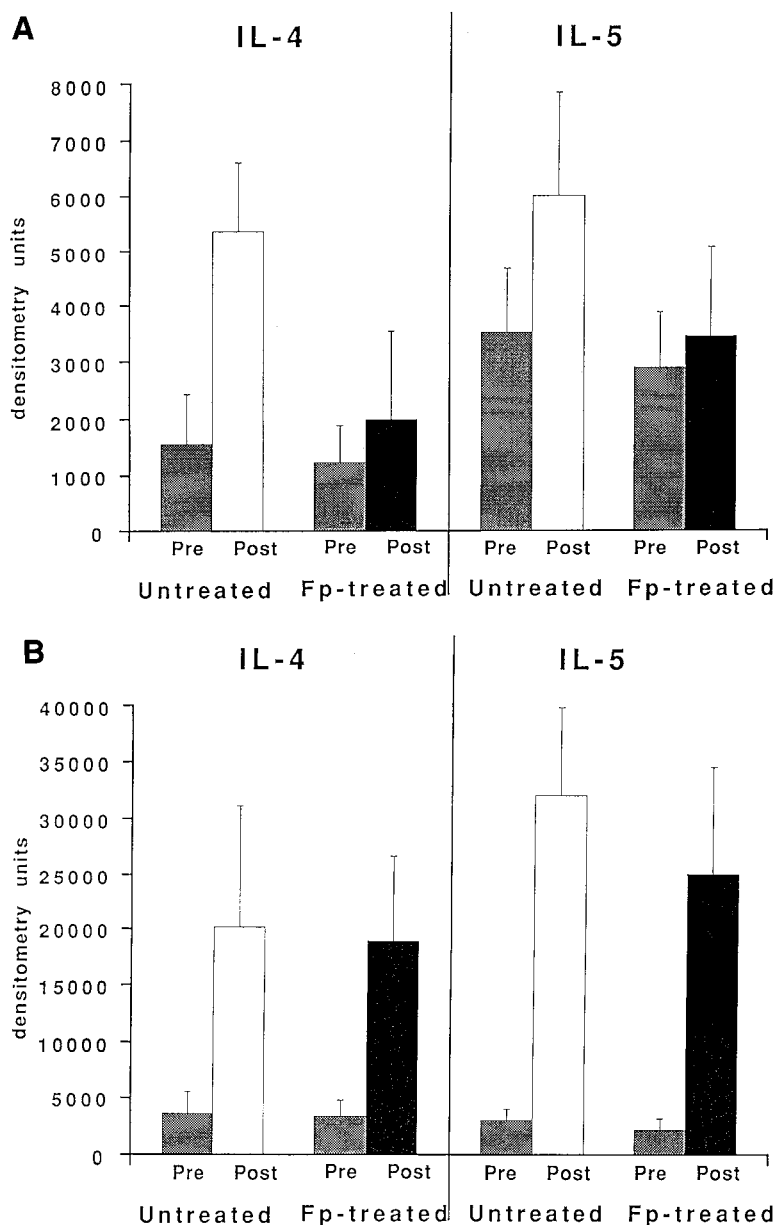


FIG. 5. The effects of fluticasone on cytokine mRNA in ragweed- and DEP-challenged subjects. (A) Expression of IL-4 and IL-5 mRNA from nasal lavages of subjects before and 18 h after challenge with 10,000 AU of ragweed. (B) Expression of IL-4 and IL-5 mRNA from nasal lavages of subjects challenged with 0.3 mg DEP. The concentrations following identical challenge following treatment for 7 days with 200 μ g of fluticasone propionate is also shown. The results are the mean densitometry units for each group ($n = 5$) +1 SD.

in a small rise in expression for a few cytokines, predominantly IL-5 (7). This small increase in cytokine mRNA elicited by ragweed challenge was absent after fluticasone treatment. This agrees with previous studies that showed that after fluticasone treatment IL-4 mRNA was markedly reduced in nasal biopsies obtained from atopic subjects challenged with allergen (16). In contrast, the large increase in cytokine production observed after DEP challenge could not be ablated

by fluticasone. Presumably, the limited inhibitory effects of fluticasone were insufficient to counteract the large polyclonal activation by the adjuvant-like activity of DEP. It should be noted that we only treated our subjects for 1 week with the approved dosing schedule (200 μ g once a day) of fluticasone. Whether longer treatment or higher doses of fluticasone would be able to block the mucosal effects of DEP remains to be tested.

Fluticasone effectively ablated the allergen-specific IgE response seen upon challenge with the model allergen ragweed. This is in accordance with previous studies that show that intranasal steroids can inhibit seasonal increases in ragweed-specific IgE observed in both serum and nasal washes from ragweed-sensitive subjects. It is interesting that in spite of this allergen-specific IgE reduction, treatment with glucocorticoid had no effect on the rise in total nasal IgE concentrations following challenge with either ragweed or DEP. This suggests that the majority of the IgE antibody produced in response to ragweed was not ragweed-specific but due to a "bystander" effect. This is in agreement with other studies that have shown that anti-birch-specific but not total IgE can be inhibited when subjects receive glucocorticoids during birch pollen season (26). The mechanisms by which glucocorticoids specifically affect the allergen-specific response is unknown. One possible explanation in this study lies in the difference between the need to generate a new anti-ragweed IgE response versus simply boosting ongoing IgE responses. The subjects, although sensitized to ragweed, did not have ongoing ragweed exposure because IgE anti-ragweed was essentially undetectable in nasal lavage prior to antigen exposure. Induction of these newly stimulated ragweed-specific T and/or B cells is likely to be more sensitive to glucocorticoid-related changes, including alterations in the cytokine microenvironment, than is the upregulation of ongoing IgE production from B cells already committed to and/or producing IgE to allergens in the subjects' environment.

In summary, our study agrees with and extends earlier studies showing that treatment with topical steroids are an effective method of lowering baseline allergic antibody production and allergic inflammation. Topical fluticasone propionate was also effective in reducing the effects of acute allergen-specific challenge. However, topical fluticasone propionate, in the doses employed, was not able to interrupt the adjuvant-like polyclonal activation of the mucosal immune system induced by DEP. These results suggest that while corticosteroid treatment should be effective in combating the effects of normal allergen exposure, other modalities are likely to be required to inhibit the increased IgE and mucosal inflammation occurring as the result of exposure to mucosal adjuvants such as DEP alone or in combination with allergens.

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