

GR β expression in nasal polyp inflammatory cells and its relationship to the anti-inflammatory effects of intranasal fluticasone

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Background: Nasal polyposis disease is an inflammatory disorder with intense eosinophilic infiltration of respiratory mucosa that is often difficult to control with topical steroids. Recent evidence suggests that overexpression of the glucocorticoid receptor splice variant GR β in inflammatory cells might contribute to steroid insensitivity in diseases such as asthma.

Objective: The purposes of this investigation were to determine whether nasal polyp (NP) inflammatory cells overexpress GR β and to examine whether GR β overexpression is associated with insensitivity to the potent topical steroid fluticasone propionate (FP).

Methods: Biopsies were obtained from 10 subjects with NPs before and 4 weeks after treatment with intranasal FP. Middle turbinates biopsies from 6 healthy, nonallergic subjects served as normal controls. Biopsies were immunostained for inflammatory cell markers as well as GR β and probed for various cytokine mRNA. The anti-inflammatory response to FP was examined in relation to pretreatment levels of GR β expression.

Results: The total numbers of inflammatory cells were increased in NPs. The percentage of inflammatory cells expressing GR β was also increased (40.5% $\pm$ 19.2% vs 16.1% $\pm$ 4.0%, $P = .009$). GR β expression in NPs was almost exclusive to T lymphocytes, eosinophils, and macrophages. An inverse correlation was observed between the baseline inflammatory cell GR β expression and the reduction after FP treatment in EG2-positive eosinophils, CD4-positive T lymphocytes, endothelial VCAM-1 expression, and IL-4 mRNA-positive cells. NPs that were "FP-insensitive" in terms of suppression of eosinophil numbers (major basic protein-positive) had a significantly greater percentage of GR β -positive inflammatory cells, a higher ratio of GR β -positive/GR α -positive cells, and increased numbers of GR β -positive eosinophils and macrophages in comparison with those that were "FP-sensi-

tive." "FP-insensitive" NPs also demonstrated a higher percentage of IL-5-positive inflammatory cells expressing GR β before and after FP treatment.

Conclusion: GR β expression appears to be a marker of steroid insensitivity in NPs. Expression of GR β by NP inflammatory cells, particularly T cells and eosinophils, might render them resistant to suppression by topical steroids and thereby contribute to persistent NP inflammation. (*J Allergy Clin Immunol* 2001;108:59-68.)

Key words: Eosinophils, sinusitis, glucocorticoid, steroid resistance, receptor

Nasal polyposis disease is a feature of chronic hyperplastic sinusitis that is characterized by eosinophil-predominant chronic inflammation.¹ The inflammation in nasal polyposis disease shares many features with chronic asthma.² Topical steroid treatment is widely advocated to control nasal polyp (NP) inflammation and growth, but the response to this treatment is often only partially successful. Clinical improvement with potent topical steroids is seen only after prolonged periods of treatment.³ A subset of subjects show progression of nasal polyposis disease despite topical steroid treatment. Given these observations, there is a need for greater understanding of the mechanisms of response of NPs to topical steroids.

Recent studies of steroid-resistant asthma have identified a potential phenotype of steroid insensitivity—namely, the overexpression of a splice variant of the glucocorticoid receptor (GR), designated GR β , in infiltrating T lymphocytes^{4,5} and bronchial epithelial cells.⁶ GR β arises from alternative splicing of the GR pre-mRNA,⁷ and GR β differs from GR α in the carboxy terminal end of the molecule in the steroid binding domain. As a result, GR β fails to bind glucocorticoids. However, heterodimerization of GR β occurs with ligand-bound GR α , and the heterodimer has been shown to bind DNA promoter regions and function as a dominant negative inhibitor of GR α .⁸ Hence it has been postulated that GR β is functionally related to steroid unresponsiveness,^{7,8} though there is not universal agreement with this tenet.⁹ Given the reported overexpression of GR β in steroid-resistant asthma, we hypothesized that a similar overexpression might be present in NP inflammatory cells and associated with steroid insensitivity.

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Abbreviations used

FP: Fluticasone propionate
GM-CSF: Granulocyte-macrophage colony stimulating factor
GR: Glucocorticoid receptor
NP: Nasal polyp
VCAM-1: Vascular cell adhesion molecule-1

We recently showed that topical fluticasone propionate (FP) caused a significant reduction in inflammatory T lymphocytes and eosinophils in NPs and suppression of the T_H2 cytokines IL-4 and IL-13.¹⁰ However, in that study, subjects were found to have a variable response to FP treatment, some showing little or no response in terms of reduction of NP eosinophils. We wished to examine whether overexpression of GR β by NP inflammatory cells might help to explain this variable response to FP. Our hypothesis was that GR β would be overexpressed in a fraction of NP inflammatory cells and that this overexpression might be associated with insensitivity to topical FP.

MATERIALS AND METHODS**Study subjects**

This study was approved by the Human Subjects Committee at Washington University School of Medicine. Subjects were recruited through advertisements and physician referrals. The 10 subjects (5 female, 5 male; mean age, 47.7 ± 12.8 years) in this study were participants in a previous double-blinded, placebo-controlled study of intranasal FP versus placebo for treatment of nasal polyposis disease.¹⁰ The present study was performed after the treatment study was completed, and only the subjects who had received active treatment with FP were included in the present study. Each subject had symptomatic chronic sinusitis with nasal polyposis, and 5 of the subjects also had clinical histories of asthma.

Systemic and intranasal steroids were withheld a minimum of 1 month and 2 weeks, respectively, before the initial NP biopsy. Beginning at the screening visit, all intranasal medications were stopped, and none were allowed throughout the study. Subjects were allowed to continue other medications, including oral decongestants, antihistamines, and medications for asthma throughout the study. No new medications were allowed from the time of the screening visit until the end of the study. The initial NP biopsies were obtained 1 week before initiation of the intranasal study drug. Each subject in the study received two 50- μ g puffs of FP per nostril twice a day for 4 weeks. Subjects were evaluated at weekly intervals during treatment. The clinical and anti-inflammatory responses to intranasal FP are the subject of a previous report.¹⁰

Normal controls

Six subjects (age, 33.7 ± 12.5 years) were recruited as normal controls. These subjects were in generally good health, and none had a history of nasal or sinus disease, allergic rhinitis, upper respiratory tract infection in the previous 6 weeks, previous sinus surgery, use of any intranasal medications, decongestants, antihistamines or oral steroids, use of intranasal cocaine, use of cigarettes in the previous 3 years, pregnancy, or ongoing exposure to animals. Nasal mucosal biopsies obtained from the middle turbinates served as the normal control tissues for immunochemistry and in situ hybridization.

Biopsies of nasal tissue

The nasal passages were decongested with 0.5% Neosynephrine, then sprayed with 4% Xylocaine solution to anesthetize the nasal mucosa. Percutaneous 2- to 3-mm biopsies of NPs or middle turbinate mucosae were obtained with 5-mm Thrucut biopsy forceps (Smith & Nephew, Memphis, TN). Generally, 2 to 4 specimens were obtained from both the right and left sides.

Tissue preparation

A portion of the biopsy was embedded in OCT, frozen in 95% ethanol plus dry ice, and stored at -70°C until processing for immunocytochemistry. Another portion of the biopsy was fixed in 4% paraformaldehyde for 24 hours, washed and stored in PBS with 15% sucrose at 4° for up to 4 weeks, and then blocked and cut at a thickness of 10 μm and processed for in situ hybridization.

Immunostaining

The details of immunostaining of NPs for inflammatory T lymphocytes, eosinophils, and macrophages have been described previously.¹⁰ The sum of CD4-positive T lymphocytes, CD8-positive T lymphocytes, major basic protein (MBP)-positive eosinophils, and CD68-positive macrophages was used to estimate the total numbers of inflammatory cells present. For colocalization studies, T lymphocytes and macrophages were stained with anti-CD3 antibody and anti-CD68 antibody, respectively—both from Becton Dickinson Corporation (San Jose, Calif). Eosinophils were stained with an anti-MBP antibody (BMK-13).

GR β immunoreactivity was detected in frozen NP tissue through use of a GR β -specific polyclonal rabbit antibody raised against human GR β (Bioaffinity Reagents, Golden, Colo). This antibody is specific for GR β and has no cross-reactivity against GR α .¹¹ The reaction was visualized through use of the avidin-biotin peroxidase complex method, as previously described.¹¹ GR α immunoreactivity was detected similarly, through use of a polyclonal rabbit antiglycocorticoid receptor α antibody (Affinity Bioreagents, Neshanic Station, NY). For negative control preparations, the primary antibody was replaced by either nonspecific rabbit immunoglobulin or Tris-buffered saline solution.

In situ hybridization

The tissues were probed for IL-5 or granulocyte-macrophage colony stimulating factor (GM-CSF) mRNA through use of in situ hybridization and antisense probes (complementary to mRNA), as previously described.¹ The specificity of the results during hybridization with antisense cRNA probes has previously been described.¹⁰ For double-labeling of cytokine-producing inflammatory cells, including IL-5-positive/MBP-positive and GM-CSF-positive/MBP-positive eosinophils, GM-CSF-positive/CD68-positive macrophages, and IL-5-positive/GR β -positive inflammatory cells, in situ hybridization was combined with immunostaining.

Quantification

For immunostaining and in situ hybridization, tissue sections were counted by a single "blinded" observer on 2 separate occasions in coded random order through use of an Olympus microscope (Tokyo, Japan) with an eyepiece reticule at 200 $\times$ magnification. The reticule was oriented along the epithelial basement membrane, and cell counts were expressed as mean counts per high-power field (0.202 mm^2). At least 2 sections were immunostained or hybridized; from these, 6 to 8 fields were counted. Results were expressed as positive cells per field. The within-observer coefficient of variation was 8% for repeated counts of immunoreactive cells and <5% for hybridized sections.

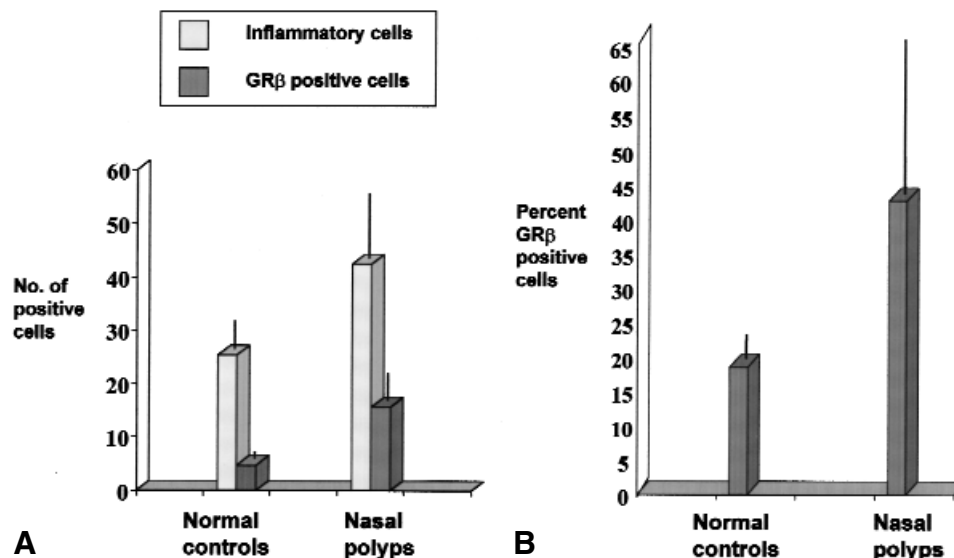


FIG 1. GR β immunoreactivity in NPs versus normal control middle turbinates. **A**, Total numbers of inflammatory cells and GR β -positive cells in NPs versus normal control middle turbinates. The differences were statistically significant ($P = .03$ and $P = .0005$, respectively). **B**, Percentage of GR β -positive inflammatory cells in NPs versus normal control middle turbinates (derived from A). The difference was statistically significant ($P = .009$).

Statistics

Each group mean was expressed as the arithmetic mean $\pm$ SD. Statistical comparisons of controls versus NPs and of "FP-insensitive" versus "FP-sensitive" NPs were performed through use of the Student *t* test. A *P* value of .05 was regarded as statistically significant in all comparisons. The relationship between expression of GR β and the response of various inflammatory markers to intranasal FP was determined by linear regression.¹²

RESULTS

The clinical improvement afforded by 4 weeks of treatment with FP was the subject of a previous report.¹⁰ Nine of the 10 subjects had an improvement in nasal peak inspiratory flow rate. Two of the 10 subjects responded poorly, as evidenced by a combination of high-level posttreatment symptoms (global symptom score, >4.0) and a worsening of global symptoms on active treatment. The most significant anti-inflammatory effects seen after FP treatment were reduction in eosinophils (8 of 10 subjects), reduction in CD4 T lymphocytes (6 of 10 subjects), reduction in IL-4 mRNA-positive cells (8 of 10 subjects), reduction in IL-13 mRNA-positive cells (9 of 10 subjects), and reduction in endothelial vascular cell adhesion molecule-1 (VCAM-1) expression (6 of 10 subjects). The levels of all inflammatory cell markers before and after FP treatment are summarized in Table I. The 2 subjects who were the symptomatic "failures" were among the 3 subjects least sensitive to FP at the tissue level (subjects 1, 5 and 7). We chose to examine further the 10 subjects treated with FP to determine whether expression of GR β in the pretreatment NP was associated with the anti-inflammatory response to FP.

GR β expression is increased in NPs

Pretreatment "baseline" GR β expression was quantified by immunostaining in 10 NPs before intranasal FP treatment and in a comparison group of 6 untreated normal control middle turbinates. Total inflammatory cells (the sum of T lymphocytes, eosinophils, and macrophages) and GR β -positive cells were increased in NPs in comparison with normal controls (42.4 ± 12.5 vs 29.3 ± 5.6 , $P = .03$; 16.1 ± 5.1 vs 4.7 ± 1.4 , $P = .0005$), as shown in Fig 1, A. The percentage of inflammatory cells expressing GR β was also increased in NPs in comparison with normal controls ($40.5\% \pm 19.2\%$ vs $16.1\% \pm 4.0\%$, $P = .009$), as shown in Fig 1, B.

GR β expression in NP is confined to inflammatory cells

GR β expression was colocalized by immunostaining to CD3-positive T lymphocytes, MBP-positive eosinophils and CD68-positive macrophages at baseline in 6 pretreatment NPs, showing a range of total inflammatory cells from 18.6 to 60.1 cells per high-power field. Nearly all (93%) of the GR β -positive inflammatory cells were accounted for by colocalization with these 3 cell types. The average distribution of GR β -positive cells was as follows: $40.0\% \pm 15.1\%$ CD3-positive lymphocytes, $23.1\% \pm 14.3\%$ MBP-positive eosinophils, $30.2\% \pm 12.4\%$ CD68-positive macrophages, and 6.7% other cells. Representative immunostaining for GR β in NPs is illustrated in Fig 2.

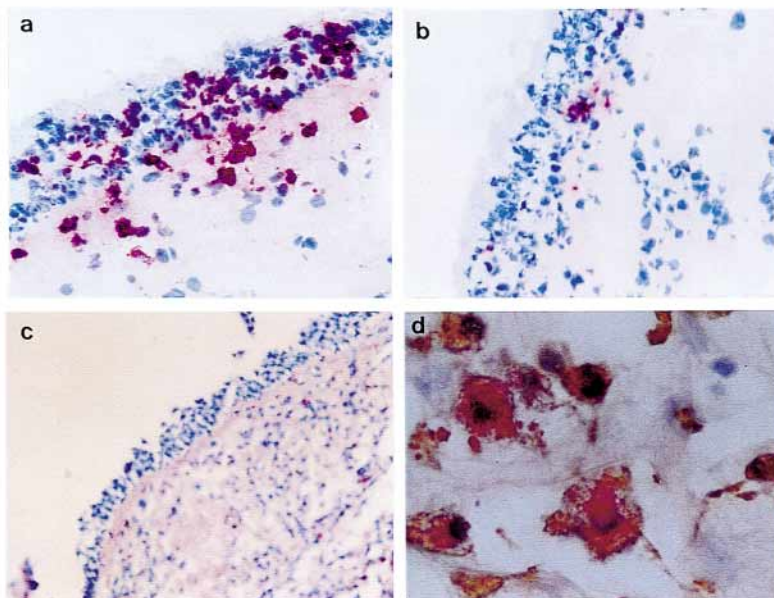


FIG 2. GR β immunostaining of nasal polyp mucosa in an "FP-insensitive" subject (a) and an "FP-sensitive" subject (b) after treatment. Note the larger number of immunoreactive cells in the "FP-insensitive" subject (a). c, A negative control for the GR β staining; an irrelevant antibody was used. d, An example of double immunostaining of GR β and CD68 in an "FP-insensitive" subject after treatment.

TABLE I. Summary: inflammatory cells, VCAM-1 expression, and cytokine mRNA levels in NP before and after treatment with fluticasone propionate

Subject no.	EG2		CD4		CD8		CD68		VCAM-1		IL-4		IL-13		IL-5		GM-CSF	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	8.3	6.1	20	17.5	2.3	4.1	13.7	21.5	3	3	4.5	3.7	19.3	14.1	9.5	8.2	12.5	10.3
2	9.3	5.3	11	10	5.5	2	17.5	10.3	3	2.5	4.0	3.5	11.5	4.7	8.2	4.5	17.3	11.2
3	10.3	2.5	20	10	7.3	3.7	6.7	12.2	2.5	2	6.2	2.5	9.7	5.1	17.1	6.9	11.2	9.6
4	14	6.1	22	15	8	2.5	8.7	7.5	4	3	5.2	2.4	10.3	4.5	2	11.5	—	—
5	3.5	5	6	9.5	2.2	4.5	6.3	13.1	1.5	2.5	1.0	2.3	18.3	13.5	9.3	9.2	10.2	11.7
6	8.1	5.3	19	7.5	4	1.5	8.5	12.5	3.5	3	6.5	3.9	12.3	7.7	16.7	12.2	14.3	13.5
7	7.1	6.3	7	12	5	3.5	11.7	17.5	2	3	4.5	5.7	15.1	17.5	12.3	7.1	13	8
8	11	7	12	12	5.2	8	16.7	12.1	3.5	3	10.1	6.7	10.3	8.1	9	12.1	13.3	15.5
9	5.1	3.3	13.5	17	2.1	6.3	3.1	4.3	2.5	2.5	6.0	5.3	12.7	8.5	15.5	9.2	17.6	11.2
10	12.3	1.5	16	10	2.2	12	10.3	6.3	4	1.5	9.0	5.7	11.3	3	20	10.1	26.7	13

Association of GR β expression with FP insensitivity in NPs

Most of the NPs demonstrated a partial suppression of inflammatory cell numbers and cytokine mRNA in response to topical FP. We wished to determine whether GR β expression in the pretreatment NP biopsies correlated with the reduction of inflammatory indices after FP treatment.

An inverse correlation was observed between the baseline percentage of inflammatory cells expressing GR β and the percentage reduction after FP treatment in EG2-positive eosinophils ($R = 0.81$; $P = .005$) and CD4-positive T lymphocytes ($R = 0.81$; $P = .005$; Fig 3). A similar relationship was observed when eosinophils were counted by means of MBP immunostaining (data not shown). No relationship was observed between baseline GR β expression and reduction in CD8-positive T lymphocytes

after FP treatment. An inverse correlation was also observed between the baseline percentage of GR β -positive inflammatory cells and the percentage reduction after FP treatment in endothelial VCAM-1 expression ($R = 0.84$; $P = .003$) and IL-4 mRNA-positive cells ($R = 0.77$; $P = .01$; Fig 3). A similar relationship was also seen with percentage reduction in IL-13 mRNA-positive cells; however, this relationship did not reach statistical significance ($R = 0.59$; $P = .07$). No relationship was found between baseline GR β expression and reduction in IL-5 or GM-CSF mRNA-positive cells (data not shown).

The inverse correlations described above suggested that GR β expression might be a marker of steroid insensitivity in NP. To examine this more closely, we compared baseline GR β expression and response to treatment in 2 subsets of subjects with nasal polyposis disease determined to be "FP-sensitive" and "FP-insensitive."

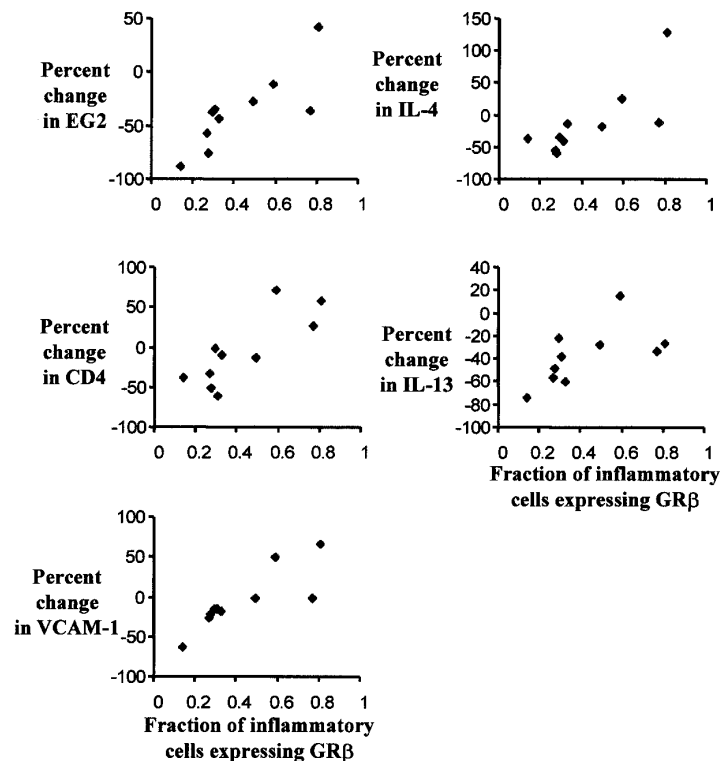


FIG 3. Linear regression analysis of the relationship between pretreatment percentage of inflammatory cells expressing GR β and the anti-inflammatory response to FP. An inverse correlation was observed between the baseline percentage of inflammatory cells expressing GR β and the percentage change in EG2-positive eosinophils ($R = 0.81$; $P = .005$), CD4-positive T lymphocytes ($R = 0.81$; $P = .005$), endothelial VCAM-1 expression ($R = 0.84$; $P = .003$), and IL-4 mRNA-positive cells ($R = 0.77$; $P = .01$). A similar relationship was seen with percentage reduction in IL-13 mRNA-positive cells; however, this relationship did not reach statistical significance ($R = 0.59$; $P = .07$).

“FP-sensitive” and “FP-insensitive” subsets of NP subjects

From the group of 10 subjects treated with FP, we identified the 3 “most sensitive” and the 3 “least sensitive” to FP on the basis of reduction of eosinophils. Within the “insensitive” group were the 2 subjects who were symptomatic failures on FP (see above). As shown in Fig 4, the differences between the “FP-sensitive” and “FP-insensitive” subjects in terms of eosinophil reduction were highly significant ($P = .004$ and $.005$, respectively). The percentage change in EG2-positive eosinophils was -73.3% in the “FP-sensitive” group and $+1.7\%$ in the “FP-insensitive” group ($P = .05$).

Reduction in NP eosinophils was arbitrarily chosen as an indicator of FP sensitivity on the basis of potential biological relevance; however, the subjects did not all show the same level of pretreatment NP eosinophils. Furthermore, there was a clear difference in the pretreatment eosinophil levels in the “FP-sensitive” and “FP-insensitive” subjects, suggesting that part of the “insensitivity” might have reflected a lack of inflammation in these subjects. We examined the other inflammatory markers listed in Table II, and again the “FP-sensitive” and “FP-

insensitive” subgroups (based on eosinophil response) segregated in terms of reductions in CD4-positive T lymphocytes and endothelial VCAM-1, with a similar trend in terms of reduction in IL-4 mRNA-positive cells (Table II). Therefore, although the pretreatment levels of inflammation were somewhat lower at baseline in the “FP-insensitive” subjects, we elected to retain these subgroups of subjects in the subsequent studies.

“FP-insensitive” NPs have an increased percentage of GR β -positive cells

The “FP-insensitive” NPs had a slightly greater number of GR β -positive inflammatory cells in the pretreatment biopsies (19.4 ± 20.3 vs 12.0 ± 26.5 , $P = .13$). However, the percentage of inflammatory cells expressing GR β was significantly higher in the “FP-insensitive” NPs ($63.0\% \pm 15.9\%$ vs $23.0\% \pm 7.7\%$, $P = .03$). In contrast, the number of inflammatory cells expressing GR α was slightly greater in the “FP-sensitive” NPs (19.3 ± 9.3 vs 15.0 ± 1.0 , $P = .14$). As a result, the ratio of cells expressing GR β /GR α was significantly higher in the “FP-insensitive” NPs (1.29 ± 0.04 vs 0.65 ± 0.10 , $P = .04$). Colocalization of GR β with T lymphocytes, eosinophils, and macrophages revealed that the 2 sub-

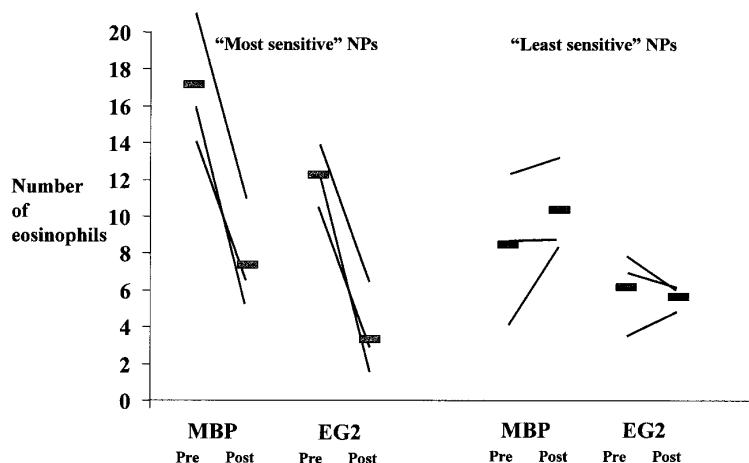


FIG 4. Comparison of "FP-sensitive" and "FP-insensitive" NPs (3 subjects per group) with respect to reduction in MBP-positive and EG2-positive eosinophils after treatment with FP. The reductions in MBP-positive and EG2-positive eosinophils in the "FP-sensitive" NPs were highly statistically significant ($P = .004$ and $P = .005$, respectively).

TABLE II. Differences in the response to treatment with FP in the "FP-sensitive" and the "FP-insensitive" NPs (N = 3 per group)

Subject group	Percentage change in inflammatory cell markers after treatment with FP*		
	CD4-positive T lymphocytes	IL-4 mRNA-positive cells	Endothelial VCAM-1 expression
FP-sensitive	-39.8% (9.3%)	-50.1% (11.9%)	-35.8% (23.2%)
FP-insensitive	39.1% (45.1%)	46.3% (75.8%)	38.9% (34.7%)
P value (for the difference)	.04	.10	.04

FP, Fluticasone propionate.

*Values shown represent means ($\pm$ SDs).

groups had equal numbers of GR β -positive T lymphocytes but that the "FP-insensitive" subgroup had greater numbers of GR β -positive eosinophils and macrophages (GR β -positive eosinophils, 6.43 ± 2.87 vs 1.69 ± 0.30 , $P = .24$; GR β -positive macrophages, 7.38 ± 1.98 vs 2.48 ± 1.02 , $P = .03$), as shown in Fig 5.

We wished to determine whether the activation status of eosinophils in NPs contributed to FP insensitivity. Hence, we looked at the suppression of "activated" versus "unactivated" eosinophils in the NPs, as shown in Fig 6. Eosinophils were regarded as activated if they were producing either IL-5 or GM-CSF.¹³ In the "FP-sensitive" subgroup, both IL-5-negative and IL-5-positive eosinophils were suppressed ($P = .03$ for both), whereas in the "FP-insensitive" subgroup, neither IL-5-negative nor IL-5-positive eosinophils were suppressed ($P = .62$ and $.61$, respectively). Similarly, in the "FP-sensitive" subgroup, both GM-CSF-negative and GM-CSF-positive eosinophils were suppressed ($P = .04$ and $.052$, respectively), whereas in the "FP-insensitive" subgroup neither GM-CSF-negative nor GM-CSF-positive eosinophils were suppressed ($P = .53$ and $.73$, respectively). Hence, eosinophil activation per se, as indicated by production of IL-5 or GM-CSF, did not appear to render the eosinophils insensitive to FP.

Similarly, we wished to determine whether the activation status of macrophages in NPs contributed to steroid insensitivity. Production of GM-CSF was used as an indicator of macrophage activation. As shown in Fig 6, neither GM-CSF-negative nor GM-CSF-positive macrophages were suppressed by FP. Hence, production of GM-CSF by macrophages appears to be a steroid-insensitive feature of NP inflammation. This might have relevance to the survival of eosinophils in NP despite FP treatment (see Discussion).

GR β expression in IL-5-producing inflammatory cells is greatest in "FP-insensitive" NPs

IL-5 has been shown to be the principal cytokine responsible for prolonged eosinophil survival within the NP tissue.¹⁴ In a previous study, we found that most of the IL-5-producing cells in NPs were T lymphocytes (67%) and eosinophils (19%).¹⁵ We wished to determine whether expression of GR β by IL-5-producing inflammatory cells rendered them insensitive to FP. The "FP-sensitive" and "FP-insensitive" NPs were double-labeled for IL-5 by in situ hybridization and GR β by immunostaining, and the numbers of IL-5-positive/GR β -positive and IL-5-positive/GR β -negative cells were counted before and after FP treatment. We found that the "FP-insensitive" NPs had a higher percentage of IL-5-posi-

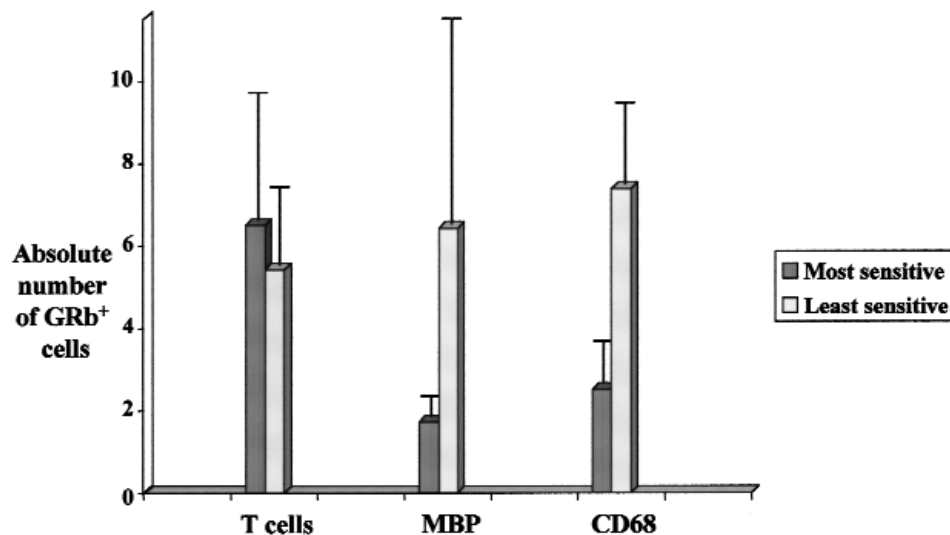


FIG 5. The absolute numbers of GR β -positive inflammatory T cells, MBP-positive eosinophils, and CD68-positive macrophages in the "FP-sensitive" and "FP-insensitive" NPs before treatment with FP. The "FP-insensitive" NPs had greater numbers of CD68+/GR β -positive macrophages ($P = .03$). The "FP-insensitive" NP also had greater numbers of MBP+/GR β -positive eosinophils, but the difference was not statistically significant ($P = .24$).

tive/GR β -positive inflammatory cells before and after FP treatment (before, $47.9\% \pm 5.0\%$ vs $31.5\% \pm 1.6\%$, $P = .006$; after, $55.7\% \pm 6.3\%$ vs $22.9\% \pm 2.7\%$, $P = .003$). In addition, the percentage of IL-5-positive/GR β -positive inflammatory cells increased after treatment in the "FP-insensitive" NPs, suggesting that these cells might in fact be relatively insensitive to FP treatment. However, in the "FP-sensitive" NPs, we found that the total number of IL-5-positive inflammatory cells and the percentage of IL-5-positive/GR β -positive inflammatory cells decreased after treatment. This suggested that at least a portion of the IL-5-positive/GR β -positive inflammatory cells were sensitive to FP treatment.

We also examined the total numbers of GR α -positive inflammatory cells in the NPs before and after FP treatment. A reduction in GR α -positive cells was seen in the "FP-sensitive" NPs (from 19.3 ± 9.3 to 13 ± 3.0 , $P = .05$). In contrast, a slight but not significant reduction in GR α -positive cells was seen in the "FP-insensitive" NPs (from 15.0 ± 1.0 to 13.2 ± 1.2 , $P = .11$).

DISCUSSION

In this study, we show that an increased number and an increased percentage of inflammatory cells in NPs overexpress the β isoform of the GR. GR β expression in NPs was found predominantly in T lymphocytes, eosinophils, and macrophages, these 3 cell types accounting for 93% of the GR β -positive cells. An inverse correlation was observed between baseline GR β expression and the anti-inflammatory effects of FP in terms of the percentage reduction in T lymphocytes, eosinophils, IL-4 mRNA-positive cells, and VCAM-1 expression after FP treatment. Furthermore, a subset of NPs that was determined to be "FP-insensitive" in terms of suppression of

eosinophils had a greater percentage of inflammatory cells expressing GR β , as well as increased numbers of GR β -positive eosinophils and macrophages in comparison with the subset that was "FP-sensitive." The percentage of IL-5-producing inflammatory cells expressing GR β was also greater in the "FP-insensitive" NPs and increased further after FP treatment. In contrast, the "FP-sensitive" NPs had greater numbers of T lymphocytes and IL-5-producing cells in their baseline biopsies and a dramatic reduction in IL-5-producing cells after FP treatment. However, in the "FP-sensitive" NPs, we did not see an increase in IL-5-positive/GR β -positive cells after FP treatment. Nonetheless, by a variety of criteria, overexpression of GR β in baseline NP inflammatory cells appears to be a marker of steroid insensitivity.

GR β has received significant attention as a potential inducible modulator of steroid responsiveness in inflammatory diseases, including asthma and ulcerative colitis.^{4,6,16} In steroid-resistant asthma, support for this hypothesis includes the observation that GR β is inducible in normal T lymphocytes by specific cytokines and the fact that a much higher percentage of T lymphocytes expresses GR β in bronchoalveolar lavage fluid than in peripheral blood from the same subjects.¹¹ The percentage of T lymphocytes expressing GR β in NPs (40%) was also clearly greater than that in normal peripheral blood (<5%), though not as great as in bronchoalveolar lavage fluid T lymphocytes in steroid-resistant asthma.¹¹ This provides further support for the hypothesis that induction of GR β expression in NP inflammatory cells can modulate the responsiveness to intranasal steroids. More importantly, induction of GR β expression might be a mechanism for steroid resistance in multiple organs.¹⁶

Several reports have indicated that overexpression of GR β modulates cellular responsiveness to steroids. Oak-

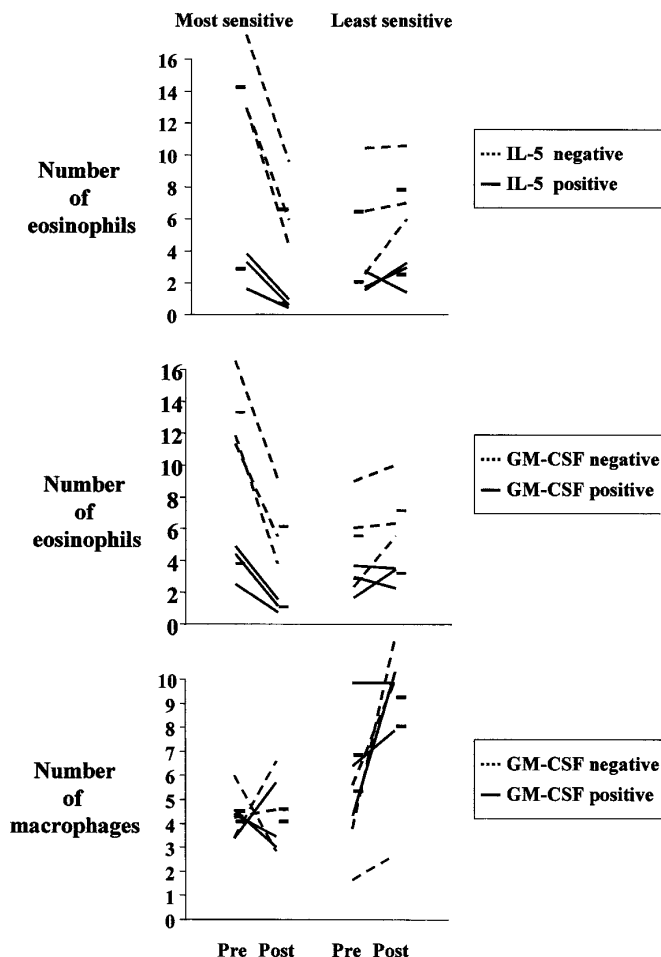


FIG 6. Relationship of eosinophil and macrophage activation status in NPs to response to FP treatment. *Top:* In the "FP-sensitive" subgroup, both IL-5-negative and IL-5-positive eosinophils were suppressed ($P = .03$ for both), whereas in the "FP-insensitive" subgroup, neither IL-5-negative nor IL-5-positive eosinophils were suppressed ($P = .62$ and $.61$, respectively). *Middle:* In the "FP-sensitive" subgroup, both GM-CSF-negative and GM-CSF-positive eosinophils were suppressed ($P = .04$ and $.052$, respectively), whereas in the "FP-insensitive" subgroup, neither GM-CSF-negative nor GM-CSF-positive eosinophils were suppressed ($P = .53$ and $P = .73$, respectively). Hence, eosinophil activation per se, as indicated by production of IL-5 or GM-CSF, did not appear to render the eosinophils insensitive to FP. *Bottom:* In the "FP-sensitive" and "FP-insensitive" NPs, neither GM-CSF-negative nor GM-CSF-positive macrophages were suppressed by FP.

ley et al¹⁷ demonstrated reduced steroid responsiveness in human COS-transformed cells after transfection with increasing amounts of a GR β -containing plasmid. In a subsequent study, they showed that GR β binding to GR-responsive elements required formation of a GR α :GR β heterodimer that binds to the GR-responsive elements and functions as a dominant negative inhibitor.⁸ Leung et al⁴ demonstrated that transfection of GR β into human T lymphocytes from normal subjects rendered them insensitive to the suppressive effects of dexamethasone on lymphocyte mitogenesis. The behavior of the GR β -transfected normal T lymphocytes mimicked that of T lymphocytes from subjects with steroid-resistant asthma, which have also been shown to overexpress GR β .⁴ Hence, it is quite likely that overexpression of GR β in inflammatory cells in NPs alters their steroid sensitivity.

We observed a significant difference in the ratio of GR β /GR α expression in "FP-sensitive" and "FP-insensitive" NPs. Although this ratio does not reflect the relative amounts of GR β and GR α in individual inflammatory cells, it is interesting that a higher ratio of GR β /GR α was seen in the "FP-insensitive" NPs, thus suggesting the possibility that the relative expression of GR β to GR α is related to steroid responsiveness in NP inflammatory cells. As mentioned above, such a relationship was demonstrated in vitro by transfecting increasing amounts of GR β into HeLa S₃ cells (which contain approximately 20,000 GR α receptors per cell).¹⁷ Furthermore, other investigators have demonstrated that GR α and GR β are coexpressed in the same cells in inflammatory lesions.⁶

We also found that fluticasone caused a reduction in cells expressing GR α in the "FP-sensitive" NPs but not

in the "FP-insensitive" NPs. This observation is similar to that of Sousa et al,⁶ who found that the ratio of GR α /GR β expression in cutaneous cell-mediated inflammatory lesions was reduced by glucocorticoid treatment in glucocorticoid-sensitive subjects but not in glucocorticoid-resistant subjects. The reason for this relative effect on GR α expression, however, is not entirely clear.

An important criterion used for steroid responsiveness was reduction of eosinophils by FP. Aside from increased expression of GR β , several other mechanisms could account for this persistence of eosinophils. One mechanism would be relatively greater local production of eosinophil survival-promoting or proinflammatory cytokines. By means of *in situ* hybridization, we found no differences between the "FP-sensitive" and the "FP-insensitive" NPs in terms of numbers of IL-4-, IL-5-, GM-CSF-, TNF- α -, or IL-1 β mRNA-positive cells. The only difference found was greater numbers of IL-13 mRNA-positive cells in the "FP-insensitive" NPs. Although the significance of this finding remains unclear, it is perhaps relevant that IL-13 has been shown to reduce the glucocorticoid receptor binding affinity in macrophages.¹⁸ This raises the possibility that IL-13 might similarly induce a change in GR phenotype in T lymphocytes or eosinophils. Another possible mechanism for eosinophil persistence in NP would be increased activation of eosinophils, as evidenced by autocrine secretion of either IL-5 or GM-CSF. We tested this possibility by examining the numbers of IL-5-positive and GM-CSF-positive eosinophils in the pre- and post-FP NP biopsies. The results indicated that IL-5 or GM-CSF production per se was unlikely to account for FP insensitivity, because IL-5-positive and IL-5-negative eosinophils were suppressed to the same degree in "FP-sensitive" NPs. Further, in "FP-insensitive" NPs, even IL-5-negative and GM-CSF-negative eosinophils failed to suppress with FP. Hence, the difference between "FP-insensitive" and "FP-sensitive" NPs did not appear to be due to a simple difference in local cytokine levels or cellular activation that we could detect. Although these arguments are by no means definitive, they raise the possibility that other factors, such as overexpression of GR β , modulate the steroid responsiveness of NP inflammatory cells.

Other mechanisms have also been proposed to account for cellular steroid resistance, including overexpression of transcription factors, such as AP-1 and NF κ B, that antagonize GR function at the level of gene transcription and via protein-protein interactions.^{19,20} AP-1 and NF κ B are induced by multiple cytokines, including TNF- α and IL-6.²¹ In patients with steroid-resistant asthma, an increase in basal AP-1 binding to DNA and reduced interaction between AP-1 and GR have been reported.¹⁹ Recently, an enhanced level of phosphorylation of JUN N-terminal kinase has also been reported, which might help to explain the increased basal AP-1 binding to DNA in steroid-resistant asthma.²² The extent to which abnormalities such as these can help to explain steroid insensitivity in nasal polyposis disease remains to be determined.

To our knowledge, this is also the first study to demonstrate GR β expression in human eosinophils. The poten-

tial effects of this overexpression on eosinophil function are intriguing. The lifespan of eosinophils in tissues is determined by a balance between survival-promoting and apoptosis-promoting signals. IL-5 has been shown to be the principal survival-promoting cytokine responsible for prolongation of eosinophil survival in NPs.¹⁴ Eosinophils are highly sensitive to induction of apoptosis by steroids, and only high concentrations of IL-5 can overdrive this apoptotic signal.²³ Hence, if induction of GR β were to reduce eosinophil sensitivity to steroids, it could theoretically alter the balance between survival-promoting and apoptosis-promoting factors, thereby prolonging eosinophil survival in tissues.

The demonstration of GR β overexpression in 30% of NP macrophages is also of potential importance. Macrophages have traditionally been regarded as relatively "steroid-insensitive" in terms of production of proinflammatory cytokines,¹⁸ but the mechanism of this insensitivity has not been elucidated. Consistent with this, we previously found that FP failed to suppress IL-1 β or TNF- α mRNA expression in NPs.¹⁰ Macrophages are clearly an important source of these cytokines. In the present study, we also observed that CD68-positive/GM-CSF-positive macrophages were resistant to suppression by FP regardless of whether they expressed GR β .

It is important to note that not all macrophage functions are glucocorticoid-insensitive. For instance, Spahn et al¹⁸ previously showed that IL-13 induces an alteration in the glucocorticoid receptor binding affinity of macrophages analogous to that which has been found in lymphocytes to be due to induction of GR β . Furthermore, they showed that this alteration had functional significance in terms of a reduced suppressive effect of hydrocortisone on LPS-induced IL-6 production. Thus the possibility remains that GR β expression in macrophages has functional significance.

Clearly, additional research is needed to elucidate mechanisms of induction of steroid insensitivity in inflammatory cells. At present, topical steroids represent the most effective therapy by which to control NP inflammation and growth. However, consistent with the results of this study, we have observed several patients whose NPs cannot be controlled by topical steroids alone and who require moderate daily doses of prednisone to maintain control of their disease. For these patients, the concept of relative steroid insensitivity appears to have merit and warrants further investigation into potential mechanisms of the effect.

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