

Allergic rhinitis and inflammation: the effect of nasal corticosteroid therapy

Fokkens WJ, Godthelp T, Holm AF, Blom H, Klein-Jan A. Allergic rhinitis and inflammation: the effect of nasal corticosteroid therapy. Allergy 1997; 52(suppl. 36): 29–32. © Munksgaard 1997.

**W. J. Fokkens, T. Godthelp,
A. F. Holm, H. Blom, A. Klein-Jan**

Department of Otorhinolaryngology, Dijkzigt
University Hospital, Rotterdam, The Netherlands

Topical corticosteroids have proved to be effective in the treatment of allergic rhinitis. The symptomatology of allergic rhinitis is considered to be the result of the accumulation and activation of inflammatory cells and cytokine release and hence the efficacy of corticosteroids is associated with their anti-inflammatory action. New advances in allergic inflammation now suggest that not only mast cells and eosinophils but also T-lymphocytes and antigen-presenting dendritic cells, play an important role in the inflammatory reaction. The effect of topical fluticasone propionate on cellular infiltration in the nasal mucosa is examined, with an emphasis on two studies performed in Rotterdam, The Netherlands. The cells influenced most by corticosteroid therapy were Langerhans' cells (antigen-presenting cells), which were almost completely eradicated, possibly resulting in diminished antigen presentation, and eosinophils. There was a reduction in the number of epithelial mast cells, but the number of T-lymphocytes only decreased following high doses of corticosteroid therapy or long-term treatment. However, T-lymphocyte function was influenced, as shown by the reduction in the T-helper₂ (TH₂)-related cytokines, interleukin (IL)-4 and IL-5. Topical corticosteroid therapy had no effect on the accumulation of macrophages. The reduction in antigen presentation, and the decrease in T-lymphocyte stimulation and cytokine production, may cause a reduced influx of eosinophils and other inflammatory cells, resulting in diminished symptomatology.

Key words: allergic rhinitis; corticosteroids; cytokines; eosinophils; Langerhans' cells; mast cells; T-lymphocytes

Dr Wytske J. Fokkens
Department of Otorhinolaryngology
Dijkzigt University Hospital
Dr Molewaterplein 40
NL-3015 GD Rotterdam
The Netherlands

Topical corticosteroids have proved to be effective in the treatment of allergic rhinitis, and have been shown to inhibit both the immediate and late-phase responses to allergen presentation. Regular prophylactic use of these agents is effective in reducing the symptoms of allergic rhinitis, including nasal blockage, rhinorrhoea, sneezing, and itching, in adults and in children (1–3). The symptomatology of allergic rhinitis is believed to be related mainly to accumulation and activation of infiltrating cells, resulting in allergic inflammation. The mechanisms involved in these cellular events include upregulation of endothelial cell adhesion molecules and an increase in a number of cytokines (e.g. interleukin-4 [IL-4] and IL-5). These cytokines may be derived from a variety of sources, including T-lymphocytes, mast cells, and eosinophils (4). Corticosteroids are able to suppress many stages of this inflammatory process. New insights into the pathophysiology of allergic inflammation have led to a number of

studies focusing on the effects of topical corticosteroids on cellular infiltrates in the nasal mucosa. This overview examines the effect of topical corticosteroids on inflammatory cells (mast cells, antigen-presenting cells, T-lymphocytes, eosinophils) and cytokines (IL-4 and IL-5) in the nasal mucosa of patients with allergic rhinitis.

Mast cells

Modern immunohistochemical staining of mast cells in nasal biopsy specimens uses monoclonal antibodies directed against neutral proteases, such as tryptase, chymase and immunoglobulin E (IgE) (5, 6). These new methods have established that the previously used technique of metachromatic staining results in gross underestimation of the number of mast cells, owing to the "ghost cell" phenomenon. Degranulation of mast cells results in their "disappearance" in nasal biopsy sections

stained with metachromatic stains such as toluidine blue and alcian blue (5). Thus, data on the effects of corticosteroid therapy on mast cell numbers in nasal biopsy sections stained metachromatically require careful interpretation.

Two studies were undertaken at Dijkzigt Hospital, Rotterdam, The Netherlands, to determine the effect of topical corticosteroids on mast cells. One study compared two doses of fluticasone propionate aqueous nasal spray (FPANS) 200 µg/day and 400 µg/day versus placebo in patients with perennial allergic rhinitis, and the other was a 2-week daily threshold allergen provocation study comparing FPANS 200 µg daily with placebo in patients with isolated grass pollen allergy. Both studies revealed a reduction in tryptase- and chymase-positive cells in the nasal epithelium after active treatment. In the provocation study, there was no significant difference between the FPANS 200 µg and placebo groups with respect to the number of tryptase- and chymase-positive cells in the lamina propria. However, in the dose-comparison perennial allergic rhinitis study, a significant reduction was observed in the lamina propria following treatment with FPANS 400 µg, but was not observed with FPANS 200 µg. This dose-dependent reduction in mast cells is in agreement with a study by Bradding et al. (7), who found a reduced influx of mast cells only in the epithelium after treatment with FPANS 200 µg, and the results described by Juliusson et al. (8) who reported a reduction of epithelial mast cells in a seasonal study using budesonide 400 µg. In a study by Rak et al. (9), a reduction in the number of mast cells in the lamina propria was achieved using FPANS 200 µg; however, this was a provocation study, in which differences are usually more pronounced than in natural disease, due to the larger allergen stimulus employed. Mast cells are now also known to contain and produce IL-4 (10, 11), which is described later in this review.

The studies suggest that the number of mast cells in the epithelium decreases after corticosteroid therapy, while higher doses are required to reduce mast cell numbers in the lamina propria. This is further evidenced by a significantly improved reduction in symptoms with FPANS 400 µg in the dose-comparison perennial allergic rhinitis study.

Antigen-presenting dendritic cells and macrophages

Antigen-presenting cells, such as Langerhans' cells (CD1⁺ dendritic cells) and other HLA-DR⁺ cells, are necessary for the presentation of allergen to T-lymphocytes. Recent animal experimental research suggests that the balance between macrophages

and dendritic cells plays a central role in the development of tolerance or hypersensitivity (12).

Langerhans' cells bind IgE to their surface by a high affinity IgE receptor (13, 14). During symptomatic allergic rhinitis, there is an increase in the number of Langerhans' cells and HLA-DR⁺ cells in the epithelium and lamina propria (15–17). An increase in macrophage numbers occurs following non-specific stimulation of the nasal mucosa, e.g. by daily lavage or brushing in experimental studies (17, 18). This increase also occurs in non-allergic controls, and care should therefore be taken not to confuse this with the specific increase caused by allergen stimulation.

A number of studies performed by our group in patients with perennial allergic rhinitis, as well as allergen provocation studies, reveal that even low doses of topical corticosteroids result in total disappearance of CD1⁺ cells in the nasal epithelium during disease, and almost complete inhibition of the influx of Langerhans' cells during allergen provocation (19–21). In addition, a significant reduction in the number of CD1⁺ cells and HLA-DR⁺ cells was observed in the lamina propria of the nasal mucosa during active disease, as well as a significant inhibition of cellular influx. In all of these studies, corticosteroid therapy did not seem to affect the number of cells of monocyte/macrophage lineage. This is in accordance with the findings of Juliusson et al. (18).

Therefore, the accumulation of dendritic antigen-presenting cells, particularly Langerhans' cells, during allergen provocation is almost totally abolished by intranasal corticosteroids, while there is no effect on the accumulation of macrophages.

T-lymphocytes

After antigen presentation by IgE⁺ Langerhans' cells, the allergen is recognized by sensitized T-lymphocytes, resulting in a cascade of regulatory cytokine-mediated events, causing induction of allergic inflammation. Activated T-helper₂ (TH₂) cells produce several cytokines, including IL-4 and IL-5, that play a role in IgE-mediated allergic inflammation (4).

In a 1-year, double-blind, placebo-controlled study in patients with perennial allergic rhinitis, biopsies were taken before and after 3 and 12 months of topical corticosteroid therapy (19). After 3 months of treatment, no significant differences were observed in the number of CD3⁺, CD4⁺, CD8⁺, or CD25⁺ cells in the epithelium and lamina propria compared with baseline values. However, after 1 year of treatment, a significant reduction in CD3⁺, CD4⁺ and CD8⁺ cells was observed in the epithelium, with no significant decrease in CD25⁺

cells (21). No significant reduction in cell numbers was found in the lamina propria. In the double-blind comparative study of two doses of FPANS in the treatment of patients with perennial allergic rhinitis, a significant reduction in CD3⁺ and CD4⁺ cells was observed in the epithelium and lamina propria with FPANS 400 µg compared with placebo (20).

In an allergen provocation study performed by Rak et al. in patients with grass pollen allergy (9), nasal biopsies were taken prior to treatment and 24 h after allergen challenge. Topical corticosteroid treatment resulted in markedly fewer T-lymphocytes and CD25⁺ (IL-2 receptor-bearing) cells in both the epithelium and lamina propria.

From these studies, it may be concluded that topical corticosteroid treatment results in a reduction in the number of T-lymphocytes and their subclasses in the epithelium. In the lamina propria, a decrease in the number of T-lymphocytes is only observed following a large stimulus (9) or a high corticosteroid dose (20).

Cytokines

Cytokines may be studied at the protein level by employing immunohistochemical staining techniques or ELISA measurements, and at the RNA level by using polymerase chain reaction (PCR) or *in situ* hybridization. Quantitative measurements of cytokine production can be made using the PCR or ELISA method, but protein staining and *in situ* hybridization studies enable co-localization of proteins and mRNA encoding for these proteins to certain cells.

In a study by Bradding et al. (7) in patients with seasonal allergic rhinitis receiving treatment with either FPANS 200 µg/day or matching placebo during the pollen season, the number of IL-4 protein-positive cells in the lamina propria was suppressed in those receiving active treatment. In contrast, FPANS treatment failed to influence the number of IL-5 and IL-6 protein-positive cells. In the double-blind comparison of two different doses of FPANS in patients with perennial allergic rhinitis (20), a significant reduction in IL-4⁺ cells was observed in both the epithelium and lamina propria after treatment with FPANS 400 µg. Using *in situ* hybridization, Masuyama et al. (22) revealed a reduction in the cells expressing RNA for IL-4, but not for IL-5, following FPANS treatment, whereas in our threshold provocation model using PCR techniques, inhibition of mRNA for both IL-4 and IL-5 was observed.

Cytokines of the TH₂ population, especially IL-4, therefore appear to be decreased by topical corticosteroid therapy. Further studies are necessary in

order to determine whether this reduction is specific for TH₂-related cytokines or whether it is a more general phenomenon.

Eosinophils

The number of eosinophils, particularly activated eosinophils, increases in the epithelium and lamina propria during symptomatic allergic disease. Eosinophils are very sensitive to corticosteroid therapy – studies performed by our group and others show a considerable reduction in eosinophils and activated eosinophils after topical corticosteroid treatment (7, 9, 20–23). The substantial decline in eosinophils is evident even if the allergen stimulus is large, e.g. in allergen provocation studies, or when the administered dose of topical corticosteroid is relatively low. This reduction in eosinophil numbers appears to be more pronounced in the epithelium than in the lamina propria, and the magnitude of this difference seems to be related to the intensity of the allergen challenge.

Conclusion

Intranasal corticosteroids influence a number of cells involved in nasal allergic inflammation, which may explain their potent effect in the treatment of nasal allergy. Data obtained by a number of investigators appear to correlate well, and any differences may be attributed to variations in allergen load, e.g. in perennial allergic rhinitis compared with allergen provocation studies, or to the varying treatment regimes employed.

The number of mast cells appears to decline in the epithelium, but only in extreme circumstances in the lamina propria. It is possible that the reduction in mediator and cytokine release by mast cells is of greater significance than the absolute reduction in mast cell numbers.

Langerhans' cells are the cells most dramatically influenced by topical corticosteroid treatment – they completely disappear – suggesting that corticosteroids have an inhibitory effect on allergen presentation. Other potential antigen-presenting cells, such as HLA-DR⁺ dendritic cells and HLA-DR⁺ lymphocytes also decrease significantly, but macrophages and HLA-DR⁺ epithelial cells are not affected by topical corticosteroid therapy, suggesting that they have a less important role in allergen presentation. So far, it has not been possible to identify which subgroups of dendritic cells, apart from Langerhans' cells, are able to present allergen to T-lymphocytes.

Following a reduction in antigen-presenting cells, the decrease in T-lymphocyte stimulation and subsequent reduction in cytokine production may

result in a reduced influx of inflammatory cells in the nasal mucosa (e.g. eosinophils), leading to a decreased inflammatory reaction and diminished symptomatology. The role of other cytokines produced by, for example, mast cells and eosinophils, requires further study.

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