

Effect of 3 months' nasal steroid therapy on nasal T cells and Langerhans cells in patients suffering from allergic rhinitis

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The effect of nasal corticosteroid therapy on allergic rhinitis is uncertain. In a double-blind, placebo-controlled study over 3 months, we investigated the influence of a new corticosteroid spray, fluticasone propionate aqueous nasal spray (FPANS), on Langerhans cells (CD1a⁺ cells), HLA-DR⁺ cells, and T cells in nasal mucosa. Efficacy was evaluated by nasal symptom score. This treatment significantly decreased the number of CD1a⁺ cells and HLA-DR⁺ cells in the nasal mucosa. Furthermore, a clear trend of decreasing numbers of T cells in nasal epithelium was found. No change in nasal symptom score was found after the treatment period. These findings suggest that fluticasone propionate aqueous nasal spray decreases the antigen presentation in nasal allergy.

Topical corticosteroids are of proven efficacy in allergic rhinitis and have been shown to inhibit both the immediate and late response to allergen (17, 18, 21, 23, 24). Topical steroids prevent the accumulation of mast cells in the nasal mucosa, as well as the degranulation of mast cells, and reduce the levels of histamine, TAME-esterase activity, and kinins in the early, late, and rechallenge allergic reactions in nasal secretions (7, 12, 21, 23). Corticosteroids have also been found to inhibit the migration of neutrophils and eosinophils (2, 14). Treatment with the corticosteroid nasal spray budesonide significantly reduces the number of eosinophils in nasal secretion of patients with perennial rhinitis (27). The operation of these drugs, however, remains uncertain.

In the skin, topically applied corticosteroids cause a decreased expression of MHC class II antigens (HLA-DR) on Langerhans cells (6). A subsequent reduction has also been reported of the number of Langerhans cells in the epidermis (22). Moreover, topical corticosteroid therapy has been claimed to decrease Langerhans cell-dependent T-cell activa-

tion, which could not be restored by exogenous interleukin (IL)-1 (4). These findings in the skin suggest that topical steroid therapy suppresses antigen presentation by a direct effect on the antigen-presenting function of Langerhans cells.

In earlier studies, we identified Langerhans cells in the nasal mucosa (11). The incidence of Langerhans cells was found to increase during allergen provocation (8, 10). Recently, activated T cells, expressing IL-2 receptors (CD25⁺) on the cell surface, were identified in nasal mucosa of patients with allergic rhinitis, suggesting a role for these cells in nasal allergy (13, 26).

For investigation of whether the number of Langerhans cells, total T cells (CD3⁺), and T-cell subsets (T-helper, CD4⁺, and T-suppressor, CD8⁺) in the nasal mucosa is influenced by corticosteroids, the effect of fluticasone propionate aqueous nasal spray (FPANS), a new corticosteroid spray, was studied in the nasal mucosa of 22 patients with perennial allergic rhinitis.

Material and methods

Patients and controls

Twenty-two patients participated in the study. The patients (mean age 26 years, 15 male, 7 female) had had perennial allergic rhinitis for at least 1 year. The diagnosis perennial allergic rhinitis was confirmed by a radioallergosorbent test (RAST) score of at least 3⁺ (3⁺–5⁺) for house-dust mite allergen (HDM) and sometimes other allergens such as dander or pollen.

Nasal biopsies

The study was conducted in a double-blind manner with patients randomized into two groups. There was a run-in period on place of 4 weeks, followed by fluticasone propionate or placebo for 12 weeks. Biopsies of nasal mucosa were taken after the run-in period and after 3 months' use of the trial drug. Nasal biopsies were taken from the lower edge of the inferior turbinate using a Gerritsma forceps and processed as described elsewhere (9).

Nasal symptom score

The symptoms of nasal blockage, nasal discharge, sneezing, nasal itching, and eye irritation were assessed by the investigator at each clinic visit and also by the patient in a diary card for a 10-day period before each visit to the clinic. Both assessments used a four-point scale.

Staining procedure

From each specimen, serial 6- μ m-thick sections of nasal mucosa were cut on a Reichert-Jung 2800 Frigocut cryostat and transferred to gelatin-coated microscope slides. The sections were stained according to a previously described immunoalkaline phosphatase (AP) method (11) using the monoclonal antibodies 66IIC7 (anti-CD1a on Langerhans cells) (Sanbio Monosan), anti-HLA-DR for dendritic cells/Langerhans cells and lymphocytes (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam), anti-CD4 (T-helper cell) (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam), anti-CD3 (total T-cell number) (Becton Dickinson, Mountain View, CA, USA), anti-CD25 (anti-IL-2 receptor on activated T cells) (Becton Dickinson, Mountain View, CA, USA), and anti-CD8 (T-suppressor cell) (Monosan, Sanbio, Uden, The Netherlands).

Cell quantification

The surface area of two sections in total, and of the epithelium and lamina propria separately, was esti-

mated by means of the image-analysis system, Videoplan Kontron 2.1. Cells binding the monoclonal antibody used had bright red surface membranes, red-stained cytoplasm, or both, depending on the cell type and/or monoclonal antibody evaluated. Cells were counted if they stained red and contained a nucleus. When a group of positive cells lay very close together or overlapped each other, only the definitely positive cells were counted.

The number of Langerhans cells and T cells was counted separately in the epithelium and the lamina propria of two sections at a magnification of $\times 250$. The number of cells/mm² section area was calculated in epithelium and lamina propria.

The number of HLA-DR⁺ cells, could not be determined reliably because many positive cells were in close proximity and/or separate dendritic cells could not be readily distinguished. The number of HLA-DR⁺ cells was assessed semiquantitatively on a scale from 0 to 4 with steps of 0.5, representing a range from practically no HLA-DR⁺ cells to large numbers of HLA-DR⁺ cells. Moreover, based on morphology and localization, semiquantitative assessment was made of the number of HLA-DR⁺ dendritic cells (DC)/macrophages, lymphocytes, and epithelial cells, also on a 0 to 4 scale. Only cells clearly resembling lymphocytes (small, round, practically no cytoplasm) were judged to be lymphocytes. The rest of the migratory cells were judged to be DC/macrophages. HLA-DR⁺ epithelial cells were easily recognized by morphology and location. Control sections treated with phosphate-buffered saline or an irrelevant monoclonal antibody were negative.

Statistical analysis

Since the frequency distribution of the number of CD1a⁺ cells and T cells per mm² in epithelium and lamina propria was not symmetric and the variances were unequal, the two-tailed Mann-Whitney rank sum test of the differences was used. Differences in the score of HLA-DR⁺ cells were analyzed with the sign test. A *P* value <0.05 was considered to indicate a significant difference between groups.

Results

The sections of nasal mucosa had an average surface area of 3 mm² and usually showed a lining of ciliated columnar epithelium with or without goblet cells and/or partially stratified cuboidal epithelium. The epithelium could not be evaluated in one patient, who was excluded from the study. The lamina propria usually consisted of a subepithelial, cell-rich layer with most of the mucous glands and a deeper collagenous cell-poor layer on the bone. All sections were deep enough to assess both layers.

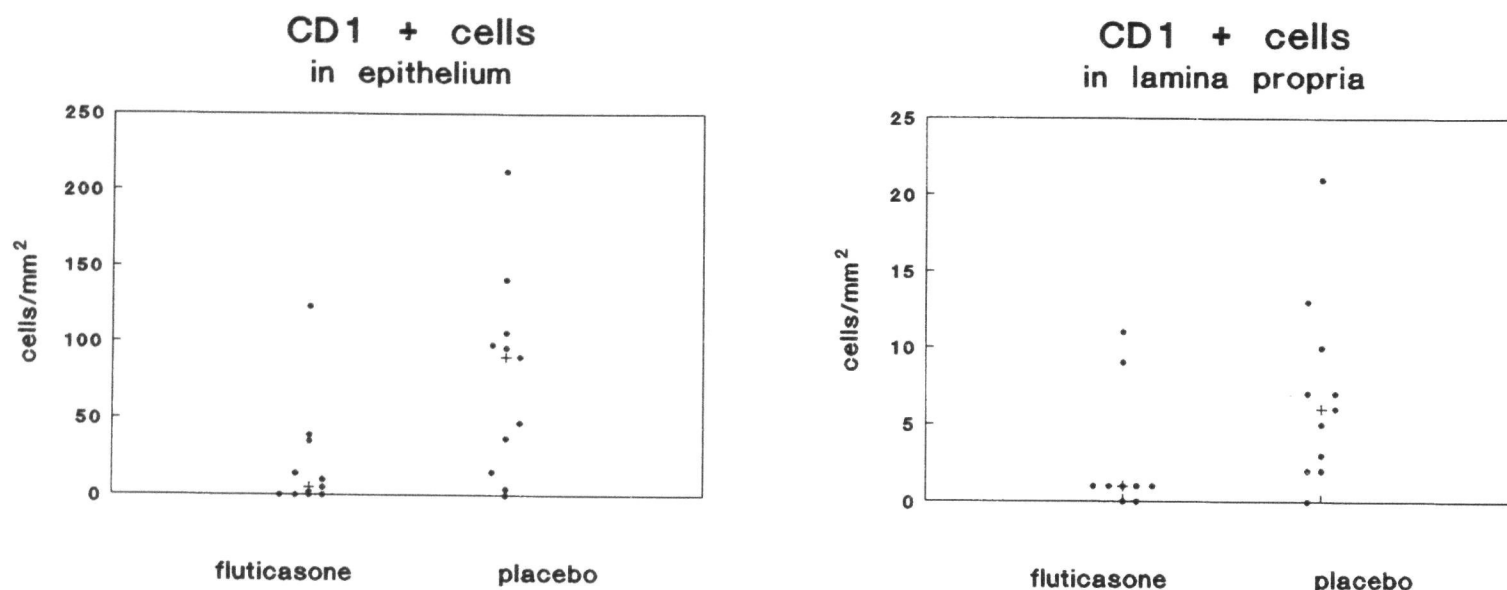


Fig. 1. Number of CD1a⁺ cells in nasal epithelium and lamina propria of patients with perennial allergic rhinitis after 3 months of either fluticasone propionate aqueous nasal spray (FPANS) or placebo therapy. Significant reduction in cell numbers in both epithelium and lamina propria was observed ($P < 0.01$) in FPANS-, but not in placebo-treated group (median number: +).

After 3 months of therapy, no histologic changes of nasal mucosa were observed in either patient group.

CD1a⁺ cells were found in the epithelium and predominantly in the subepithelial layer, in and around the glandular tissue of the lamina propria. All sections showed CD1a⁺ dendritic cells in the epithelium and in the lamina propria before the start of the trial. After 3 months of therapy, the number of CD1a⁺ cells in epithelium and lamina propria (Fig. 1) in the group receiving fluticasone propionate was significantly lower than in the placebo group (epithelium $P < 0.01$; lamina propria $P < 0.01$).

HLA-DR⁺ cells were found in the epithelium and predominantly in the subepithelial layer of the lamina propria. The HLA-DR⁺ migratory cells showed mostly dendritic and sometimes lymphocytic mor-

phology. Epithelial cells in the epithelium and/or epithelial cells in the mucous glands were positive in 13 out of 44 sections evaluated. Occasionally, clusters (100–1000 cells) of HLA-DR⁺ cells (dendritic and lymphocytic morphology) were seen in the lamina propria, mainly in the subepithelial layer.

Before the trial, no significant difference was found in the total score of HLA-DR⁺ cells, or the score of HLA-DR⁺ cells with dendritic or lymphoid morphology between the placebo group and the group about to receive FPANS. However, the score of HLA-DR⁺ cells differed significantly between the patients. After 3 months of therapy, the group receiving FPANS showed a significant decrease in the score of the HLA-DR⁺ cells with a dendritic morphology (epithelium $P < 0.01$; lamina propria $P < 0.01$), the score of the HLA-DR⁺ cells with

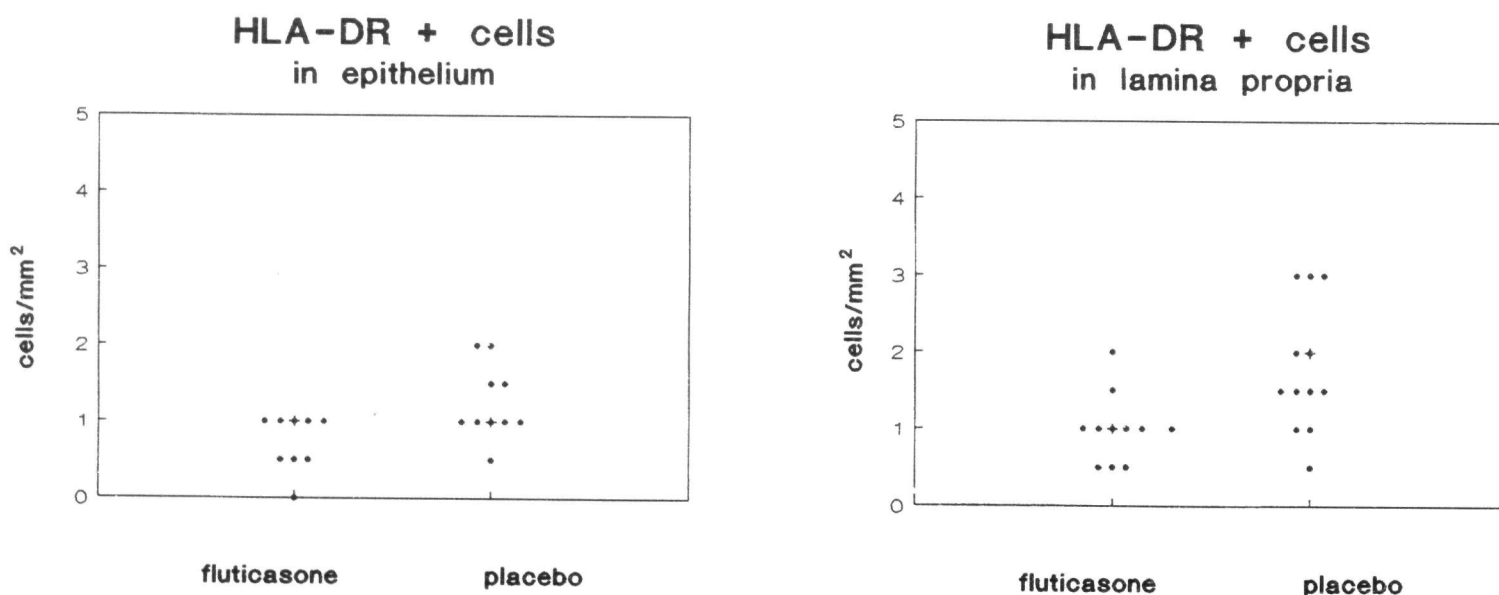


Fig. 2. Score of HLA-DR⁺ cells, counted semiquantitatively, in nasal epithelium and lamina propria of patients with perennial allergic rhinitis after 3 months of either fluticasone propionate aqueous nasal spray (FPANS) or placebo therapy. Significant reduction in cell numbers in both epithelium and lamina propria was observed ($P < 0.001$) in FPANS-, but not in placebo-treated group (median number: +).

a lymphocytic morphology (epithelium $P < 0.05$; lamina propria $P < 0.005$), and the total number of HLA-DR⁺ cells in epithelium ($P < 0.001$; lamina propria $P < 0.001$), whereas the placebo group did not do so (Fig. 2).

T cells were mainly observed in the lower and middle layer of the epithelium. In the lamina propria, most T cells were seen in the subepithelial layer and between the glands. No significant change in number of total T cells, T-cell subsets, and activated T cells was found after corticosteroid therapy in nasal mucosa (Figs. 3 and 4). In nasal epithelium of patients treated with fluticasone propionate, a clear downward trend of both CD3⁺ and CD8⁺ T cells was observed, although no significant difference with placebo-treated patients was found ($P = 0.06$ and $P = 0.07$, respectively).

Symptom score as assessed by the diary card did not show a significant difference in total symptoms score or separate symptom score between the FPANS-treated group and the placebo-treated group during the treatment period (data not shown).

Discussion

In this study, the influence of a new nasal corticosteroid, i.e., fluticasone propionate, on nasal Langerhans cells, T cells, and HLA-DR⁺ cells was investigated. Nasal corticosteroid therapy has become an established procedure in allergic rhinitis. The exact working mode, however, is unclear. In studies on biopsies of nasal mucosa, in contrast to those on the skin, so far no significant structural changes have been found following local corticosteroid therapy (15, 19).

In the skin, topical corticosteroids reduce the number of Langerhans cells and the expression of HLA-DR on these cells. Possibly due to the decreased HLA-DR expression, the antigen-presenting capacity of Langerhans cells was reduced in mice, and possibly also in man (1, 4). These findings suggest that topical steroid therapy suppresses antigen presentation by a direct effect on the antigen-presenting function of Langerhans cells.

In this study, we evaluated the number of CD1a⁺

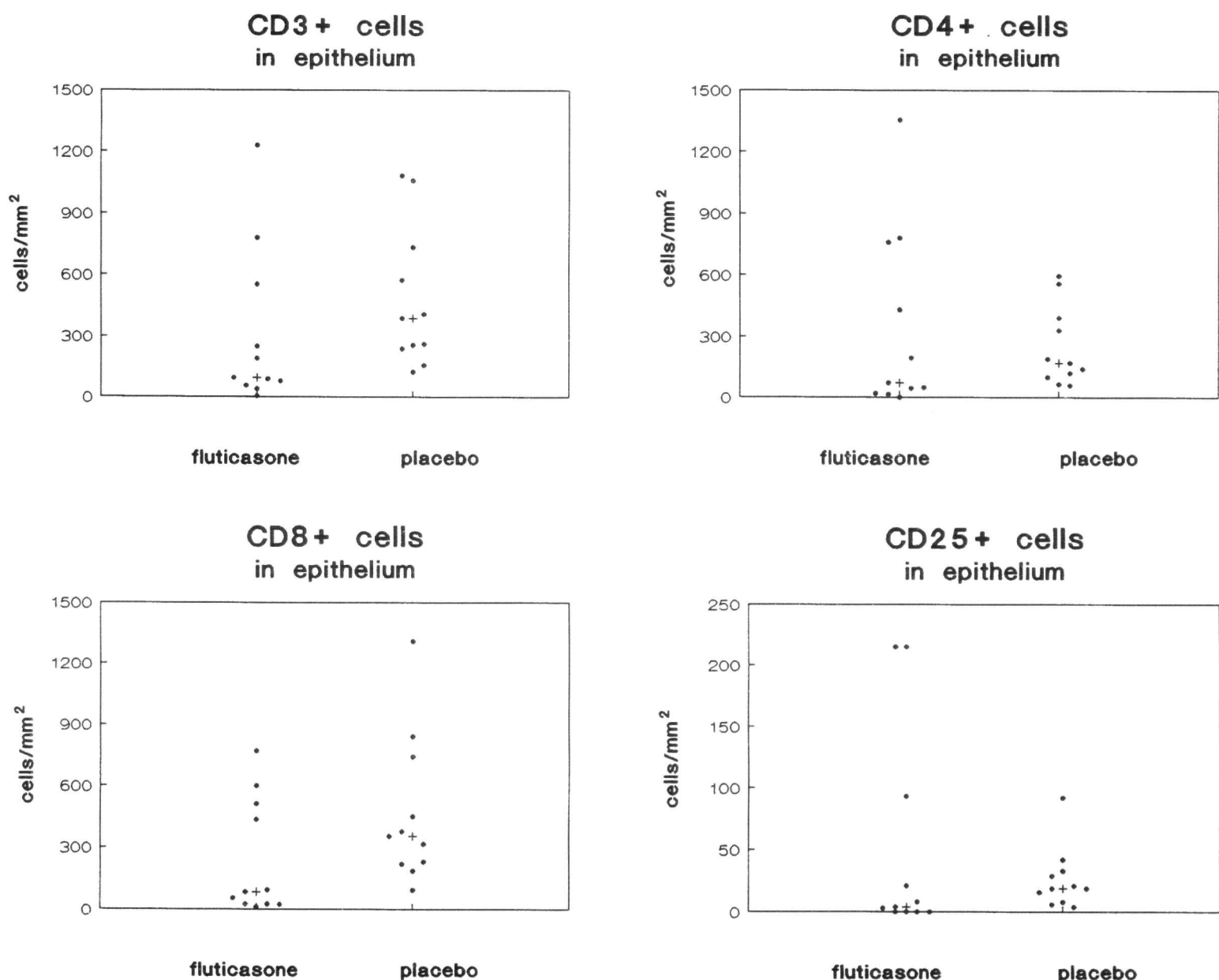


Fig. 3. Number of T cells and subsets in nasal epithelium of patients with perennial allergic rhinitis after 3 months of either fluticasone propionate aqueous nasal spray or placebo therapy. No significant change in cell numbers was observed (median number: +).

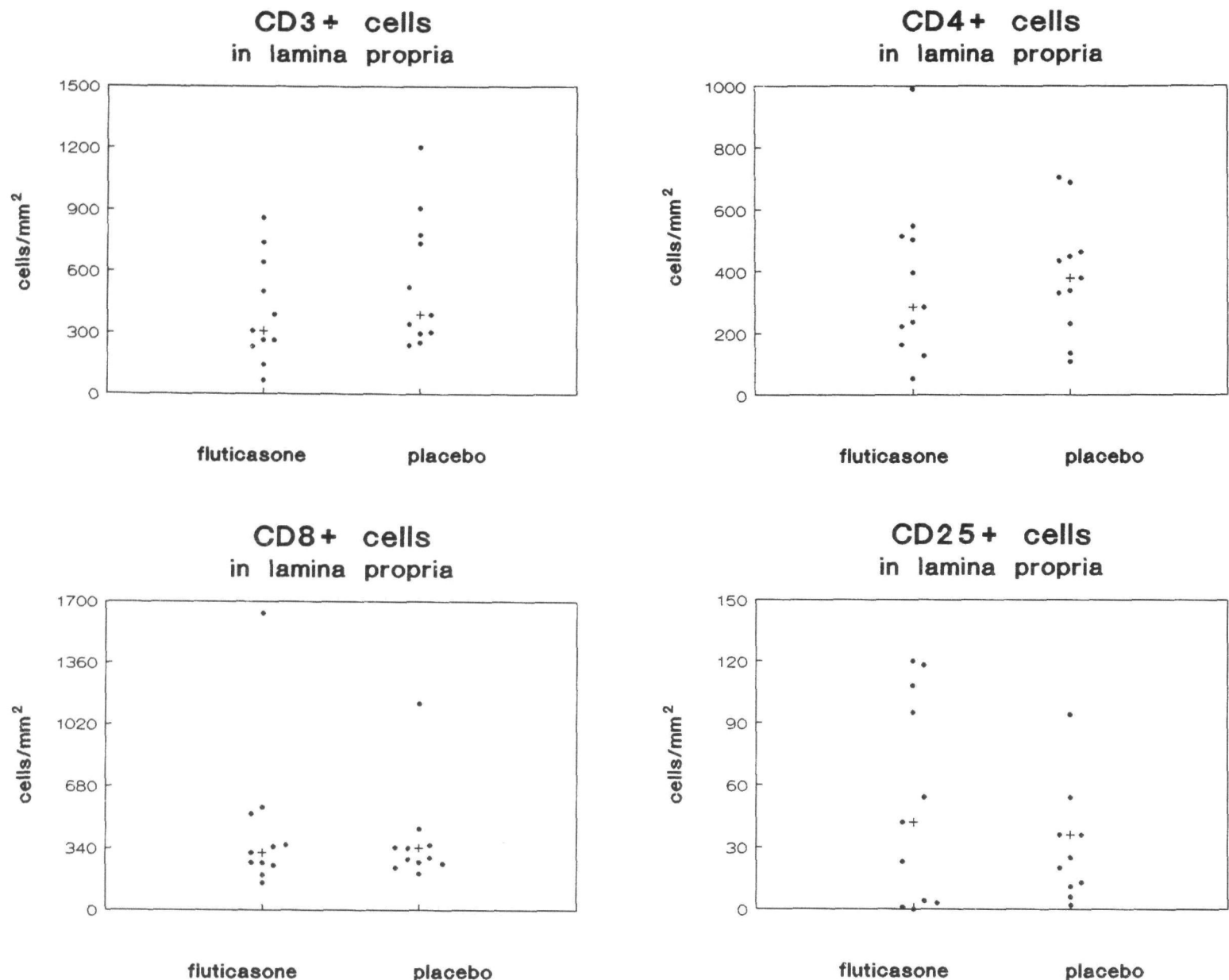


Fig. 4. Number of T cells and subsets in lamina propria of patients with perennial allergic rhinitis after 3 months of either fluticasone propionate aqueous nasal spray or placebo therapy. No significant change in cell numbers was observed (median number: +).

cells and HLA-DR⁺ cells in the nasal mucosa of patients with perennial allergic rhinitis before and 3 months after fluticasone propionate therapy. The score of HLA-DR⁺ cells decreased significantly during therapy.

In the skin of the guinea pig, Belsito et al. described a loss of Ia (MHC class II) but no irreversible structural changes during glucocorticosteroid treatment (5). In the human skin, selective reduction in the expression of HLA-DR was also found, but not of CD1a (6, 22). These results suggest a change in cell surface markers rather than loss of the whole cell. However, studies in the human skin also show a decrease of CD1a⁺ cells following more prolonged (a few days) glucocorticosteroid treatment. In the literature, no EM studies of human skin could be found to prove definitely that not only does the expression of CD1a decrease, but the number of CD1a⁺ cells as well.

In this study, we found a considerable decrease in number of CD1a⁺ cells in the epithelium during fluticasone propionate therapy (median numbers from 127 to 5). This decrease is much larger than the

decreases reported in the skin. Possible explanations are that the glucocorticosteroid therapy is more effective in the nose, probably due to better penetration, or that the new FPANS is a stronger glucocorticosteroid. In the skin, the decrease in the number of CD1a⁺ cells was dependent on the potency of the topical corticosteroid used (3).

In vitro, corticosteroids have been known to reduce T-cell responses to mitogen (16). IL-2 receptor expression does not seem to be affected after corticosteroid therapy (1). During the late-phase response, a reduction of the influx of mononuclear cells in the nose after pretreatment with flunisolide was seen (20). However, no data are available concerning the dynamics of T cells in nasal mucosa in the continuous allergic reaction. In our study, no significant change in number of T cells, T-cell subsets, and activated (IL-2R⁺) T cells in nasal mucosa was found after 3 months of corticosteroid therapy, a result which apparently corresponds with the *in vitro* findings.

The efficacy of FPANS has been proven in the treatment of allergic rhinitis (17). In our study, no

significant reduction in symptom score was found. The patients in this study were selected on the basis of having a RAST score of at least 3⁺. Some patients had serious, while others had only mild, nasal complaints. This, in combination with a relatively small patient group, probably explains why FPANS failed to relieve the symptoms in this study.

In conclusion, the present study shows a decrease in CD1a⁺ cells and HLA-DR⁺ cells, but no significant change in T-cell and activated T-cell numbers, in nasal mucosa of patients with perennial allergic rhinitis during FPANS therapy. This finding suggests a decrease in antigen presentation. The subsequent decrease in T-cell stimulation, without a corresponding decrease in IL-2 receptor expression, may result in a reduction of the reactions that are dependent on T-cell-derived mediators. Further study is necessary to determine whether Langerhans cells disappear from the nasal mucosa during FPANS therapy, or whether these findings are a result of a change in cell surface markers. Moreover, the effect of FPANS therapy on antigen presentation of Langerhans cells and T-cell function has yet to be evaluated.

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