Abstract. Chronic rhinosinusitis (CRS) is one of the most common chronic diseases. The etiology and classification of CRS, with and without nasal polyps, remain unclear. Eosinophils and their products are important in the pathophysiology of allergic diseases and in host immunity to certain organisms. Interleukin 13 (IL-13) plays a pivotal role in eosinophilic inflammation. The migration of epithelial cells requires permanent re-establishment of the intercellular connection. Intercellular connections are maintained by the modulation of adherens junctions consisting of an E-cadherin/β-catenin complex. In our study we examined the eosinophilic and non-eosinophilic paranasal mucosa obtained from two patients undergoing functional endoscopic sinus surgery. Cell cultures were incubated with human recombinant IL-13 for up to 72 h and β-catenin concentration was determined with ELISA techniques. Furthermore, immunostaining for β-catenin was used for the semi-quantitative description of specimens. We were able to ascertain a significant increase in β-catenin expression in the eosinophilic paranasal cell culture after IL-13 administration compared to the non-eosinophilic culture. Immunostaining for β-catenin was restricted to the membrane of the cells. Concerning the increased mural expression of β-catenin, we presume that a fibrotic reaction similar to asthma and chronic obstructive pulmonary disease occurs in patients suffering from CRS. Furthermore, β-catenin overexpression might be responsible for mucosal thickening and IL-13 seems to be an important marker in eosinophilic CRS.

Introduction

Chronic rhinosinusitis (CRS) is one of the most common chronic inflammatory diseases, and has been defined as a disease of the nasal and paranasal sinus mucosa persisting for more than 3 months, with mucosal changes ranging from inflammatory thickening to nasal polyps (1). Accumulations of activated eosinophils within tissues are the hallmark of this condition; however, the etiology and pathophysiology of CRS are poorly understood (2). Activated eosinophils contribute to nasal polyp pathology by virtue of basic protein deposition, epithelial disruption and basement membrane denudation, and through production of inflammatory mediators and cytokines (3,4). CRS is frequently observed in patients with asthma, and the two diseases have a number of common pathological features: the activation of T-helper type 2 (Th2)-like lymphocytes and eosinophils secreting interleukin (IL)-3, IL-5, IL-13, eotaxin and granulocyte-macrophage colony stimulating factor (GM-SCF). In consequence some authors describe CRS as the ‘asthma of the upper airways’ (2,5). In the establishment of nonallergic CRS, IL-8, which is generated by neutrophils and mucosal epithelia, has been reported to play a pivotal role (6). Tokushige et al proposed that IL-1 production by neutrophils induced the expression of the intercellular adhesion molecule (ICAM-1) on endothelial cells, leading to neutrophil infiltration in CRS (7). It has been demonstrated that the peripheral blood mononuclear cells (PBMCs) of patients suffering from CRS produced large amounts of IL-13 when exposed in vitro to Alternaria species (8). No similar findings were noted with PBMCs from non-diseased individuals. In CRS, patients showed increases in both Th1 (IFN-γ) and Th2 (IL-5, IL-13) immune responses directed toward Alternaria but no shift in the Th1/Th2 balance (8). In contrast, an increased Th2 and decreased Th1 imbalance has been reported to play a key role in the pathogenesis of asthma (9). In CRS, collaborative effects of Th1 and Th2 immune responses to fungi may be responsible for the inflammation and remodeling of sinonasal airways.

The adherens junctions of keratinocytes are composed of cadherin, β-catenin, α-catenin and p120 (10-14). These substructures guarantee the integrity and maintenance of organized
which is expressed selectively on Th2 cells. Ligation of (MDC) are ligands for the CC chemokine receptor 4 (CCR4), chemokine (TARC) and macrophage-derived chemokine methasone (19). This decrease was inhibited by the addition of dexamethasone (19).

The cytochemokines (CC) thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) are ligands for the CC chemokine receptor 4 (CCR4), which is expressed selectively on Th2 cells. Ligation of TARC/MDC and CCR4 plays an important role in the migration of Th2 cells into inflamed tissues (20,21). Immunohistologic staining has revealed that keratinocytes express TARC and MDC in the lesional, but not in the nonlesional, skin of atopic dermatitis in vivo (22-24). In a nontumorigenic human HaCaT keratinocyte cell line the synthesis and secretion of TARC has been observed after stimulation with TNF-α (25). In HaCaT keratinocyte cell lines, IL-4/IL-13 stimulation reduced staining for E-cadherin and catenins (26). Thus, IL-13 may enhance the internalization of the adherens junction complex, which leads to a down-regulation of TARC/MDC production.

We hypothesized that IL-13 administration might increase b-catenin expression in CRS cell culture. This study was designed to analyse the expression of b-catenin contributing to cell-cell adhesion in CRS tissue. We describe the expression of b-catenin in CRS in comparison with non-eosinophilic paranasal sinus mucosa.

Materials and methods

Tissue collection and culture of human chronic rhinosinusitis epithelial cells. All CRS cells were obtained from 4 patients suffering from CRS and undergoing functional endoscopic sinus surgery at the Department of Otorhinolaryngology at the University of Mannheim, Germany in 2006. Prior to surgery, written consent was obtained from all patients for the use of tissue samples of the resected paranasal mucosa and turbinate. After surgery the tissue samples were examined by a pathologist and diagnosed as eosinophilic CRS depending on the amount of eosinophilic granulocytes in the samples. We set up an epithelial culture of the IL-13 and untreated culture cell lines was analysed. Concurrently, cultured CRS cells were incubated with different levels of IL-13 (1-6 ng/ml) for up to 72 h.

Immunohistochemistry. Immunohistochemical analysis was performed using a monoclonal mouse anti-human antibody directed against b-catenin (C19220, Transduction Laboratories, Lexington, KY, USA). Immunostaining was performed using the alkaline phosphatase-anti-alkaline phosphatase method (APAAP). The sections received a microwave pre-treatment, which required boiling for 15 min at 600 W using citrate buffer for b-catenin. The following steps were performed using an automated staining system, Dako TechMate 500 (Dako, Hamburg, Germany). The sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibody 1:300 for b-catenin. Slides were rinsed once in the buffer (Puffer für APAAP). The sections received a microwave pre-treatment, which required boiling for 15 min at 600 W using citrate buffer for b-catenin. The following steps were performed using an automated staining system, Dako TechMate 500 (Dako, Hamburg, Germany). The sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibody 1:300 for b-catenin. Slides were rinsed once in the buffer (Puffer Kit, Dako). Immunoreaction was demonstrated with the Dako ChemMate Detection Kit (APAAP, mouse, code no. K 5000), according to the specifications of the manufacturer. The sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibody 1:300 for b-catenin. Slides were rinsed once in the buffer (Puffer Kit, Dako). Immunoreaction was demonstrated with the Dako ChemMate Detection Kit (APAAP, mouse, code no. K 5000), according to the specifications of the manufacturer. The sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibody 1:300 for b-catenin. Slides were rinsed once in the buffer (Puffer Kit, Dako). Immunoreaction was demonstrated with the Dako ChemMate Detection Kit (APAAP, mouse, code no. K 5000), according to the specifications of the manufacturer. The sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibody 1:300 for b-catenin. Slides were rinsed once in the buffer (Puffer Kit, Dako). Immunoreaction was demonstrated with the Dako ChemMate Detection Kit (APAAP, mouse, code no. K 5000), according to the specifications of the manufacturer. The sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibody 1:300 for b-catenin. Slides were rinsed once in the buffer (Puffer Kit, Dako). Immunoreaction was demonstrated with the Dako ChemMate Detection Kit (APAAP, mouse, code no. K 5000), according to the specifications of the manufacturer. The sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibody 1:300 for b-catenin. Slides were rinsed once in the buffer (Puffer Kit, Dako). Immunoreaction was demonstrated with the Dako ChemMate Detection Kit (APAAP, mouse, code no. K 5000), according to the specifications of the manufacturer. The sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibody 1:300 for b-catenin. Slides were rinsed once in the buffer (Puffer Kit, Dako). Immunoreaction was demonstrated with the Dako ChemMate Detection Kit (APAAP, mouse, code no. K 5000), according to the specifications of the manufacturer. The sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibody 1:300 for b-catenin. Slides were rinsed once in the buffer (Puffer Kit, Dako). Immunoreaction was demonstrated with the Dako ChemMate Detection Kit (APAAP, mouse, code no. K 5000), according to the specifications of the manufacturer. The sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibody 1:300 for b-catenin. Slides were rinsed once in the buffer (Puffer Kit, Dako). Immunoreaction was demonstrated with the Dako ChemMate Detection Kit (APAAP, mouse, code no. K 5000), according to the specifications of the manufacturer. The sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibody 1:300 for b-catenin. Slides were rinsed once in the buffer (Puffer Kit, Dako). Immunoreaction was demonstrated with the Dako ChemMate Detection Kit (APAAP, mouse, code no. K 5000), according to the specifications of the manufacturer.

Analysis of b-catenin-immunostaining. The rate of expression was analysed semi-quantitatively. The number of positively marked epithelial cells was graded 0 (no positive
cells), 1 (<20% positive cells), 2 (20-50% positive cells) and 3 (>50% positive cells). The intensity was noted as I (faint) or II (strong). The combination of these immunohistochemical reaction patterns resulted in 7 possible scores: 0, 1/I, 2/I, 2/II, 3/I, and 3/II. The reaction scores 0 to 2/I were classified as negative or low expression and 2/II to 3/II as high expression of ß-catenin.

**Statistical analysis.** We used the t-test in order to evaluate the p-value (p<0.05) of differences between cultures treated with IL-13 and the control groups. The influence of the various IL-13 concentrations and incubation time was analysed using the GLM procedure.

**Results**

**Incubation up to 72 h with IL-13.** Regarding the total protein concentration, after 3 days of incubation with IL-13, the levels of ß-catenin increased. After 8 h of incubation with 1 ng/ml IL-13 in epithelial cell culture of eosinophilic paranasal mucosa, the average concentration of ß-catenin was 18.235 ng/ml. The levels of ß-catenin after 24 h in the same eosinophilic cultures were 19.0825 ng/ml. Increase of ß-catenin concentration was ~5%. After 48 h, the further increase was 3% (19.59 ng/ml). The total concentration of ß-catenin reached a maximum after 72 h of incubation, 20.0225 ng/ml (increase of 2%), whereas in the control the concentration of ß-catenin decreased ~7%. The percentage of ß-catenin from the total protein concentration increased from 0.008 to 0.010% (Table I).

At 6 ng/ml IL-13, the average ß-catenin concentration in eosinophilic culture was 16.48 ng/ml after 8 h of incubation and reached a maximum of 21.8825 ng/ml ß-catenin after 72 h of incubation. However the percentage of ß-catenin from total protein showed an identical increase of 0.008 to 0.003% for the incubation with 1 and 3 ng/ml IL-13. The GLM procedure showed no significant influence of the concentration of IL-13 on the total ß-catenin concentration (p=0.124) (Fig. 1A-D).

However, the incubation with IL-13 showed a significant difference in the ß-catenin concentration of the eosinophilic paranasal tissue culture compared to the control (p<0.0001). In non-eosinophilic paranasal tissue culture the ß-catenin concentration was not significantly different for incubation with 1 to 6 ng/ml IL-13 (p=0.7016).

Comparing total protein content and the fraction of ß-catenin at different points in time in the eosinophilic cultures of inferior turbinates, the concentration of ß-catenin increased in cultures of 1 ng/ml (21.8875 ng/ml, 0.007%), 3 ng/ml (20.1975 ng/ml, 0.006%) and 6 ng/ml (24.2675 ng/ml, 0.008%) respectively. The expression of ß-catenin was significantly different in eosinophilic turbinate cultures compared with the control culture (p<0.0001). The non-eosinophilic turbinate culture also revealed a significant difference in ß-catenin concentration in comparison to the control group (p=0.0401).

Further statistical analysis was performed in order to evaluate the influence of IL-13 concentration, eosinophils and hours of incubation. The analysis of variance following the GLM procedure revealed a significant influence of eosinophils (p<0.0001) and incubation time (p<0.0001) in eosinophilic paranasal tissue. In terms of IL-13, the concentration of IL-13 failed to show significant influence on the ß-catenin concentration in these cultures (p=0.4373) (Table I).

**Immunohistochemistry.** The basal layer showed a high reactivity for ß-catenin. The immunostaining was restricted to the membrane of the cells. Comparing the immunoreactivity...
Figure 1. β-catenin-percentage of total protein. Results of the ELISA (incubation with 6 ng/ml for 8, 24, 48 and 72 h). (A) Culture of paranasal eosinophilic CRS. (B) Culture of non-eosinophilic paranasal CRS. (C) Culture of inferior turbinate eosinophilic CRS. (D) Culture of non-eosinophilic inferior turbinate CRS.

Figure 2. (A) Immunohistochemical illustration of the inferior turbinate (hematoxylin and eosin). (B) Immunohistochemical activity against β-catenin in the inferior turbinate. (C) Immunohistochemical analysis of β-catenin activity in a culture of non-eosinophilic CRS without IL-13 application and (D) in culture of paranasal eosinophilic CRS after 24 h of incubation with 6 ng/ml IL-13.
of the basal layer of non-eosinophilic paranasal mucosa and eosinophilic paranasal mucosa in CRS there was no difference in β-catenin expression. However, the eosinophilic paranasal mucosa samples showed a high mural expression of β-catenin. The results are presented in Fig. 2A-D and Table II.

Discussion

β-catenin was identified as a crucial link in the cadherin-catenin complex (10). Transducting via the intracellular component α-catenin by arranging the binding to cytosolic filaments and the extracellular component E-cadherin, β-catenin affects cell adhesion in epithelial tissue (15). It has been reported that the migration of inflammatory cells reduces the intensity of immunofluorescence of E-cadherin in cultured human nasal epithelial cells (27). However, the authors reported that the degree of decrease did not differ in eosinophils compared to the decrease induced by neutrophils. Furthermore, the degranulation extracts from stimulated eosinophils were not able to induce a change in E-cadherin expression by themselves. Nevertheless, a loss of epithelial E-cadherin was observed after infiltration through eosinophils (28). The effect of cell transmigration on the expression of cadherins and catenins in tissue-resident cells was reported in the relation between polymorphonuclear leucocytes and vascular endothelial cells (29). E-cadherin and β-catenin are potent adhesion molecules and blockage of these adhesion molecules induced tissue destruction in the observed tissue (30). Notably, β-catenin is considered as a physiological protein guaranteeing integrity of the epithelial tissue formation. However, it is also considered as a tumour-enhancing factor in certain conditions (15). In previous studies, we observed an up-regulation of mural β-catenin concentration through antisense treatment in external auditory canal cholesteatoma in vitro (31). We were able to up-regulate β-catenin by incubation with IL-13. Notably, the cytoplasmatic concentration of β-catenin did not change in comparison to non-eosinophilic paranasal mucosa samples. As CRS and bronchial asthma have a number of pathological features in common, some authors describe CRS as the ‘asthma of the upper airways’ (2,5). CD4+ Th2 lymphocytes and their cytokine products, especially IL-4, IL-5, IL-9 and IL-13, are essential for generating such asthmatic landmarks as eosinophil inflammation, hyperplasia and subepithelial fibrosis. Among Th2 cytokines, IL-13 is considered particularly critical. For example some authors report that the local application of IL-13 can induce the asthma phenotype in non-immunized mice (32,33). Notably, glucocorticoids are not able to suppress IL-13-induced airway hyperresponsiveness and goblet cell hyperplasia (32). IL-13 has a variety of proinflammatory effects that are relevant to CRS and bronchial asthma, including the ability to induce IgE production and endothelial cell VCAM-1 expression, whereas vascular cell adhesion molecule (VCAM-1) plays an important role in eosinophil migration in inflamed airways (34). In general, the natural variation in the coding region of IL-13 seems to be an important genetic determinant of susceptibility to allergy (35).

In conclusion, we provide evidence that application of IL-13 increases the β-catenin expression in human eosinophilic paranasal mucosa cell culture. Immunohistochemistry revealed a restriction of β-catenin to the membrane of the cells. In our study, we were able to examine the relations between eosinophilic inflammation and nasal tissue remodelling. Further studies will be necessary to investigate the cellular nature of remodelling inductors and the basic pathways of initial airway inflammation. Thus further molecular links between the cytokines of Th2 lymphocytes and the E-cadherin/β-catenin complex might be revealed.

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References


