

Transepithelial migration of activated eosinophils induces a decrease of E-cadherin expression in cultured human nasal epithelial cells

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Abstract

Background The damage of respiratory epithelium in allergic diseases has a close correlation with the extent of eosinophil infiltration. It seems to be a good possibility that eosinophil infiltration could induce the changes in the expression of the epithelial cell adhesion molecules, which play a key role in the maintenance of structural and functional rigidity of epithelium.

Objective We observed the expression of E-cadherin in cultured human nasal epithelial cells (HNECs) to study whether could it be affected by transepithelial migration of inflammatory cells, especially eosinophils.

Methods *In vitro* study of the transmigration assay was designed using various types of inflammatory cells and HNEC monolayers. Various assays of each experimental group were done under the stimulation of interleukin-5 (IL-5) and/or platelet activating factor (PAF). Subsequently immunohistochemistry for E-cadherin was performed in the HNECs. The intensity of immunofluorescence of E-cadherin was quantified using confocal laser scanning microscopy (CLSM) system and compared before and after the transmigration.

Results The mean intensity of immunofluorescence for E-cadherin decreased significantly after the transmigration of any types of inflammatory cells. Above all, the migration of eosinophils treated with IL-5 and PAF had an eminent effect on the decrease, whereas the degranulation extracts derived from eosinophils activated by IL-5 and secretory IgA (sIgA) did not affect the intensity.

Conclusion This work suggests that transepithelial migration of inflammatory cells can directly induce the decrease in epithelial E-cadherin expression. Furthermore, the most prominent change was induced by transmigration of activated eosinophils, which might be caused by some mechanisms independent of the eosinophil contents. The decrease in E-cadherin expression may trigger the damage of epithelial barrier, which contributes to the pathogenesis of allergic diseases.

Keywords: E-cadherin, eosinophil, epithelial damage, nasal allergy, transmigration

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Introduction

Allergic disorders of the respiratory tract, such as allergic rhinitis [1,2] and bronchial asthma [3,4], are pathologically

defined by destructive changes of the airway epithelium and eosinophil accumulation from capillaries into the airway lumen. The damage of respiratory epithelium is known to contribute significantly to the pathogenesis of these diseases, which induces injury of the barrier function and change in permeability of mucosa [5–8]. In asthma, histological investigations have demonstrated that severe

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damage of the bronchial epithelium is closely correlated with the extent of eosinophil infiltration [9]. Many studies have shown that some of eosinophil products have the potential to cause epithelial damage and submucosal oedema in airways [10–13]. Thus, eosinophils are considered to play an important role in the pathogenesis of asthma [14–16].

It is well known that the eosinophils observed at the site of allergic inflammation have been previously activated during their migration process, on which various chemoattractive mediators have a great influence [17,18]. In particular, the platelet activating factor (PAF) is one of the dominant and potent chemoattractants, with a wide range of biochemical activities [13,19–21]. Interleukin-5 (IL-5), a representative cytokine in allergic inflammation, is known to have eosinophil-selective chemoactivities; it facilitates the chemotactic response, the release of granule proteins, and the production of interleukins and other substances of eosinophils [22–24]. In the final process of migration from the blood vessels to the airway lumen, eosinophils penetrate and pass through the intercellular spaces of epithelial cells and the tight junctions [13,25]. Furthermore, airway epithelial injuries and/or damages may require the contact between eosinophils and epithelial cells or eosinophil penetration [26,27]. Several papers concerning the transmigration of eosinophils in airway epithelium have been made in the past few years [26,28]. However, the precise mechanism of transepithelial migration as it is linked with epithelial damage is still unknown.

In a recent study involving a guinea-pig model of nasal allergy [25], we have reported the effects of local antigen challenge *in vivo* on the distribution of E-cadherin, which is widely recognized as a representative cell adhesion molecule playing a key role in the maintenance of the structural and functional rigidity of epithelium [6,29–31]. Using immunohistochemistry, we observed a local decrease of E-cadherin expression closely associated with eosinophils infiltrated in the antigen-challenged epithelium [25]. However, the mechanism by which eosinophils induced the decrease in the expression of E-cadherin in epithelial cells was unclear. Therefore in the present study we designed an *in vitro* study using the transmigration assay [27,32] to determine if the transepithelial migration process of inflammatory cells, especially that of eosinophils, might contribute to the epithelial injury. We observed the expression of E-cadherin in the cultured human nasal epithelial cells (HNECs) monolayer and studied whether the expression could be affected by the transepithelial migration of inflammatory cells. The quantitative analysis of the E-cadherin expression was performed by measuring the intensity of immunofluorescence staining for E-cadherin, using a confocal laser scanning microscope (CLSM) system [33]. We then compared the intensity after the

transepithelial migration of some types of inflammatory cells, including eosinophils activated by IL-5 and/or PAF.

Materials and Methods

HNECs specimen procurement, preparation and culture

Turbinal mucosa samples were collected from three male patients without atopic disease. The patients had moderate to severe nasal obstruction. Inferior turbinal mucosa samples were obtained at the time of surgery for submucosal resection. Informed consent was obtained after adequately explaining the objective and procedures of the present study.

The method of HNECs purification was modified from that of Altman *et al* [34]. The surgically removed specimens were rinsed three times in Ca^{2+} - and Mg^{2+} -free PBS containing 500 U/mL penicillin, 500 $\mu\text{g/mL}$ streptomycin, and 6 $\mu\text{g/mL}$ fungizon. The epithelium was peeled away from most of the underlying connective tissue, cut into approximately 4×4 mm square pieces, placed in PBS containing 0.05% collagenase (type II) and 1% ethylenediaminetetraacetic acid (EDTA), and incubated at 37 °C for 1 h. The epithelium was then separated from any remaining connective tissue and placed in trypsin/EDTA for 10 min at 37 °C. The epithelial pieces were pipetted vigorously for 5 min, and the resulting cell suspension was transferred to a centrifuge tube. Fresh media containing 10% foetal calf serum (FCS) was added to inactivate the trypsin, and 5 min of vigorous pipetting was repeated. The cell suspension were pooled and centrifuged at 300g for 5 min. The cell pellet was placed into Dulbecco's modified Eagle's medium (DMEM) containing 20% foetal bovine serum in a culture dish after the specimens had been washed three times with PBS.

The cells were then placed onto 60-mm plastic culture dishes coated with type I-rich collagen (Sigma Chemical Co, St. Louis, MO, USA) and incubated at 37 °C under a humidified atmosphere of 5% CO_2 and 95% air. After incubation for 1 h, the medium was discarded, and the plate was washed three times with DMEM to remove floating cells. The HNECs were cultured in a MCDB153 (Life Technologies, Grand island, NY, USA) medium containing 10 ng/mL epithelial cell growth factor and 1% bovine pituitary body extract. The cultures of HNECs were fed twice weekly and subcultured twice or three times before 70–80% confluence. Morphological observations were performed to confirm that the cells consisted primarily of epithelial cells. More than 95% of the HNECs showed positive reactions with antihuman cytokeratin antibody MNF116 (Dakopatts, Glostrup, Denmark), which was shown to react with a wide range of cytokeratins including keratins nos. 5, 6, 8, 17 and 19.

Isolations of human neutrophils, mononuclear cells, and eosinophils

Human neutrophils were isolated from normal heparinized peripheral blood samples with the standard techniques of Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden) gradient separation, dextran sedimentation, and hypotonic lysis of erythrocytes [35]. The samples were collected from five healthy volunteers, who were free from allergic diseases and blood eosinophilia; in their peripheral blood, the percentage of eosinophils in the leucocyte fraction was less than 1%. Preparations were over 95% pure as determined by Wright stain and over 98% viability as determined by trypan blue exclusion. Human mononuclear cells were isolated from healthy donors by the method as previously described [36]. The neutrophils and mononuclear cells were washed twice in PBS and resuspended in RPMI 1640 medium (Life Technologies) with 10% FCS, separately.

Human eosinophils were isolated from peripheral blood obtained from five patients with allergic rhinitis (three men and two women, average age 25.0 ± 7.8 years). The diagnosis of allergic rhinitis was based on a typical history of perennial symptoms of allergic rhinitis, eosinophilia in nasal smears, and a positive radioallergosorbent test to house dust and/or mites. The leucocyte fractions were obtained by dextran sedimentation as described above. Eosinophils were then isolated by negative immunomagnetic selection using CD16 microbeads and the MACS system (Miltenyi, Biotec Inc, Germany) [37]. The resulting eosinophil purity was 98.7 ± 3.8 as determined by microscopic examination with Hinkelman staining. For this study we used eosinophils in which the viability (based on the trypan blue test) was more than 98%.

Activating eosinophils with IL-5

The isolated eosinophils were divided into half; one half was pretreated with IL-5 before the migration assay, and the other half was treated with PBS as a control. IL-5 stock solution was added to the eosinophil-rich suspension at the final concentration of 100 pg/mL and then incubated at 37°C under a humidified atmosphere of 5% CO₂ and 95% air for 18 h. After the incubation, the cells were washed in PBS three times and suspended in RPMI-1640 medium with 10% FCS.

Production of supernatant of eosinophil extracts

The supernatant of eosinophil extracts was obtained by a modification of the method of Fujisawa [38]. In brief, purified eosinophils were resuspended in medium at required cell numbers, incubated with 100 pg/mL of IL-5 for 1 h at 37°C, after which the secretory IgA (sIgA)-coated Sepharose 4B beads (Pharmacia Fine Chemicals,

Piscataway, NJ, USA) were added to the cell suspension. Subsequently, eosinophils and beads were incubated for 4 h at 37°C. This procedure of eosinophil stimulation has been apparently demonstrated to induce eosinophil degranulation [38]. After the incubation, the mixture of eosinophils and beads was centrifuged at 1200g for 10 min. The supernatant was collected and stored at -20°C until assayed.

Transepithelial migration assay

The transmigration of the inflammatory cells through the HNECs was investigated using 24-well tissue culture treated Transwell plates [32]. After two to three passages, the HNECs were cultured directly onto collagen type I-coated, Transwell culture inserts (6.5-mm diameter polycarbonate membrane filters with 5.0-µm pores, Costar Co, Cambridge, MA, USA). They were checked by phase-contrast microscopy. At confluence, the HNECs on membrane filters of Transwell culture inserts were put into the lower compartments filled with a MCDB153 medium. In all of the migration experiments, the upper compartments of the chamber inserts were washed twice with the medium before the addition of inflammatory cells into these compartments.

The inflammatory cells prepared by the process mentioned above were used in the transmigration experiments immediately without long preservation; they were added to each of the upper compartments ($7-10 \times 10^5$ cells/well). The medium alone was placed in the upper compartment as a control. To study the chemotactic potential of PAF and its effect on epithelial damage, PAF was added into the lower compartments (below the filters) at a final concentration of 3×10^{-7} M. The medium alone was placed in lower compartments as a control in each experiment. The plates were incubated at 37°C under a humidified atmosphere of 5% CO₂ for 1 h.

After the incubation period, the inserts were collected, rinsed three times in PBS, and taken for histological preparation. Each content in the lower chambers were collected to count the cell number, which had transmigrated from the upper compartments through the HNEC monolayers and pored membrane filter. The count was done under a light microscope with a haemocytometer. The transmigration rate (TR) was determined as follows [26]:

TR (%) = (the number of cells collected from the lower compartment/the number of cells added to the upper compartment) $\times$ 100.

As the combinations of inflammatory cells from five individuals and HNECs from three patients, 15 transmigration assays were performed for each experimental condition.

The supernatant of eosinophil extracts was placed into the upper components in place of the inflammatory cells, and the plates were incubated at 37 °C under a humidified atmosphere of 5% CO₂ for 1 h.

Immunohistochemistry

After the migration period, carefully collected inserts with the HNEC monolayers were rinsed three times in PBS and subsequently fixed by immersion in a fixative (4% paraformaldehyde in 0.1 M PBS) for 15 min. Before application of the primary antibody, the specimens were blocked for 1 hour with 0.02 M tris buffer saline (TBS) containing 0.01 M CaCl₂, 5% goat serum and 3% bovine serum albumin, and then incubated overnight at 4 °C with the rat monoclonal antibody to E-cadherin (ECCD-2, Takara Bio, Shiga, Japan; 1:200). The specimens were washed in TBS and then incubated for 2 h at room temperature with fluorescein-isothiocyanate (FITC)-conjugated antibodies to rat IgG (Cappel, Organon Technika, Durham, NC, USA; 1:200). After washing in TBS, the membrane filters with the HNEC monolayers were carefully removed from the Transwell inserts to be mounted on slides.

Immunohistochemical specificity controls were obtained by replacing the primary antibody to the rat IgG protein (Zymed, San Francisco, CA, USA), and omitting the first antibody.

CLSM and quantitative immunohistochemical evaluation

The quantitative analysis to evaluate E-cadherin immunoreactivity was modified from the previously described method of Gazzaley *et al.* [33], by which relative difference in immunofluorescence intensity levels was quantified. After the process of E-cadherin immunohistochemistry, the HNEC monolayers on Transwell filter membranes were picked up and mounted on slides. For each condition of the transmigration, 15 sample slides were prepared for observation. The slides were studied using a confocal laser scanning microscope (LSM 410, Carl Zeiss Co Ltd, Oberkochen, Jena, Germany) by an investigator who was blinded throughout the evaluation as to which slides were from which experimental group. The confocal parameters were established at the beginning of the study and remained constant throughout the analysis to yield unbiased measurements for each set of comparisons.

Five fields were randomly selected on each section, and scanned by the CLSM at the same predetermined z-axis distance from the surface of the slides. Each digitized image of square fields consisted of 512 × 512 × 8 bit pixel array, in which every pixel was assigned a grey level intensity value ranging from 0 to 255, were picked

up. An image-analysing program NIH Image (version 1.55; a public domain image processing and analysis program for the Macintosh) was used to determine the average pixel intensity of each field.

Analysis of colocalization of E-cadherin and eosinophils

To observe the colocalization of E-cadherin immunoreactivity and the transmigrating eosinophils, some of the eosinophils were labelled with the fluorescent probe 3,3'-diiodoacetylindocyanine (DiI) (Molecular Probes Inc, Oregon, USA) [39] before the transmigration assay. The eosinophils suspended in 1 mL of RPMI-1640 were incubated with 40 µL of DiI stock solution (2 mg DiI dissolved in 1 mL ethanol solution) at 37 °C for 1 h. The viability of the eosinophils, based on the trypan blue test, was shown to not change significantly after this treatment for DiI labelling. The marked eosinophils were washed twice with RPMI-1640, and then added to the upper compartment of the Transwell. The Transwell insert was collected after the 1 h assay, and taken into the process of immunofluorescence staining for E-cadherin as described above. The sections were inspected and scanned within the double-label channel in CLSM, with the focus of z-axis changed little by little gradually distance from the surface to the bottom of the HNEC monolayers.

For electron microscopy, the collected membranes were immersed in 2.5% glutaraldehyde and 2% paraformaldehyde in a 0.1-M phosphate buffer (pH 7.4) overnight. After washing and subsequent postfixation in 1% osmium in a phosphate buffer, block staining were performed with 1% uranyl acetate in 50% ethanol solution, followed by dehydration through an ascending series of ethanol solutions, passage through propylene oxide, and embedding in EPON 812. The ultrathin sections were stained in uranyl and lead salt solutions.

Statistical assessment

Results were calculated as the mean ± SE for each experimental condition tested. Differences between experimental groups were compared using Student's *t*-test for independent means, and a statistical difference was defined as one with a *P*-value of <0.05.

Results

Characterization of the HNECs cultures

Inverted phase contrast microscopy of the HNECs cultured on the type I collagen-coated filters revealed a monolayer of polygonal cells that were nearly homogeneous

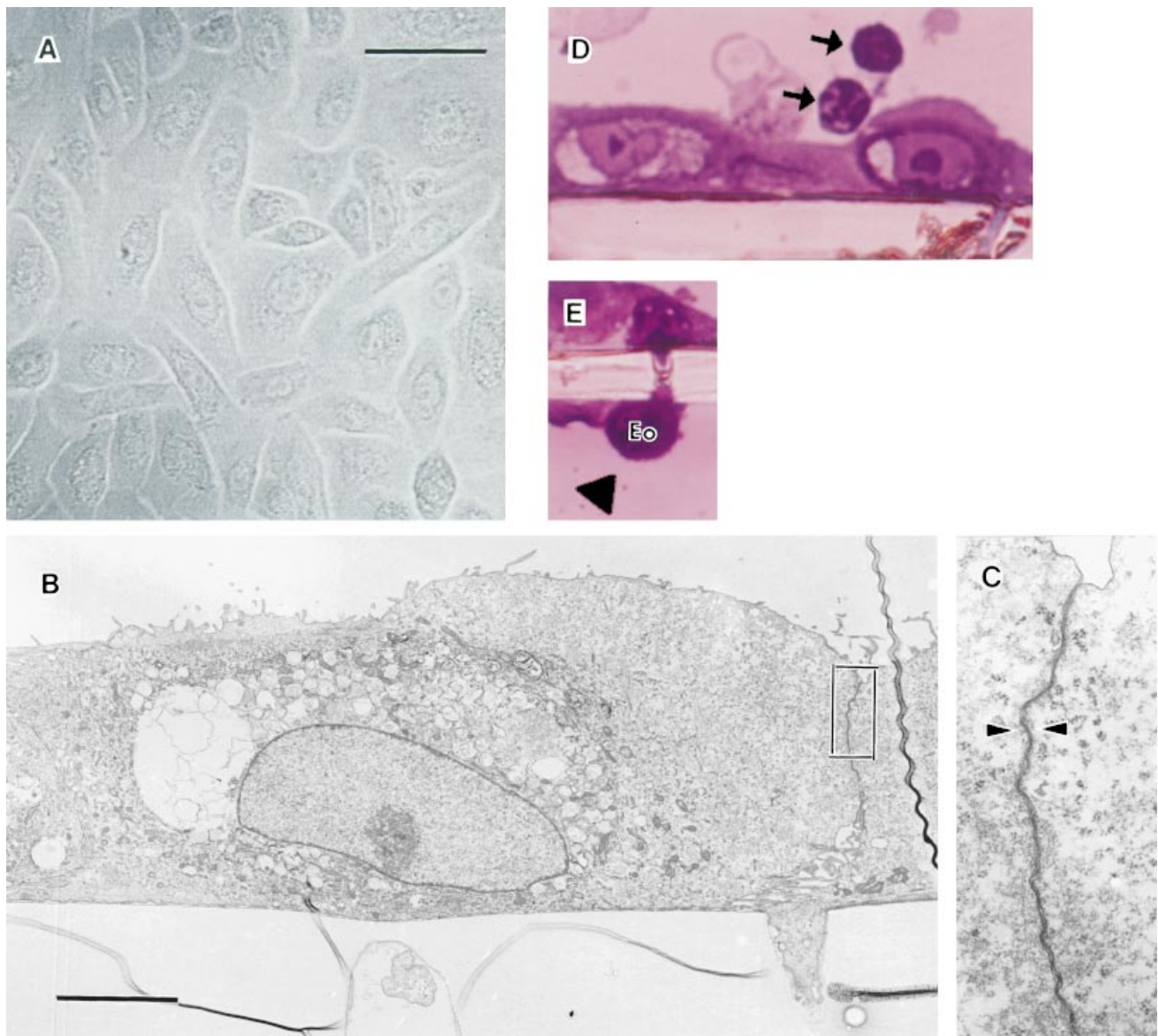


Fig. 1. Histological sections of human nasal epithelial cells (HNECs) cultured on the type I collagen-coated filters and transmigrating eosinophils. (A, B). Inverted phase contrast microscopy and electron microscopy reveal a monolayer of polygonal cells. (C) The boxed area in (B) at higher magnification confirms intimately close contact between HNECs (arrowhead). (D, E) Eosinophils added to the upper compartment (arrows) pass through the intercellular space of the HNECs monolayer, next through the 5.0- μ m pores of the filter, and then fall into the lower compartment (arrowhead). Scale bars = 50 μ m (A); 5 μ m (B).

in shape and density (Fig. 1A). Electron microscopy revealed that the lateral membranes of the adjacent HNECs were close enough to each other to form cell-to-cell contact (Fig. 1B,C). The eosinophil added to the upper compartment passed through the intercellular spaces of the HNECs monolayer, next through 5.0- μ m pores of filter and was falling into the lower compartment (Fig. 1D,E).

Chemotactic effect of IL-5 and PAF stimulation on the transepithelial migration of inflammatory cells

The mean transmigration rate (TR) of the eosinophils, neutrophils and mononuclear cells through the HNEC monolayers for 1 h were $14.8 \pm 1.30\%$, $15.8 \pm 1.34\%$ and $9.1 \pm 1.05\%$, respectively, in the nontreated control samples. The changes of TR induced by the stimulation

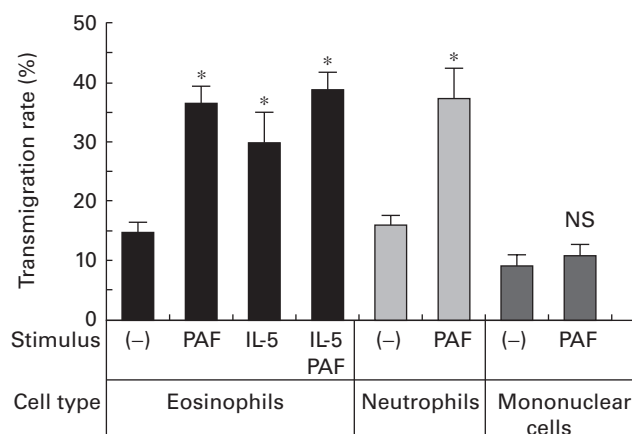


Fig. 2. The transmigration rate (TR) of eosinophils, neutrophils, and mononuclear cells through HNEC monolayers. The pretreatment of eosinophils with IL-5 significantly increased the TR of the eosinophils. The existence of PAF in the lower compartment throughout the migration induced a significant increase in the TR of eosinophils and neutrophils, but not in the TR of mononuclear cells. * $P < 0.05$. Mean $\pm$ SE.

of IL-5 pretreatment and/or PAF are shown in Fig. 2. The pretreatment of eosinophils with IL-5 significantly increased the TR of the eosinophils (2.10-fold) compared with a control treated with PBS. The PAF-induced chemotactic effect in the assay was measured for each type of inflammatory cells. With the presence of 3×10^{-7} M of PAF in lower compartment throughout the assay, the TR of the eosinophils and neutrophils increased by 2.51-fold and 2.40-fold, respectively, which were statistically significant. On the other hand, the effect of PAF on the mononuclear cell transmigration not significant.

Immunohistochemistry

In the specificity control experiment, there was no sign of nonspecific labelling (data not shown). In the control HNEC monolayers without inflammatory cells transmigration, E-cadherin immunoreactivity was seen continuously along the adjacent epithelial cell borders (Fig. 3A). In the HNEC monolayers following the transmigration of the eosinophil or neutrophil, on the other hand, the immunoreactivity appeared greatly weakened throughout the field, and indeed was not detected in some areas (Fig. 3C,D). The decrease of the E-cadherin was seen all over the HNEC monolayers on Transwell filters after the migration assay

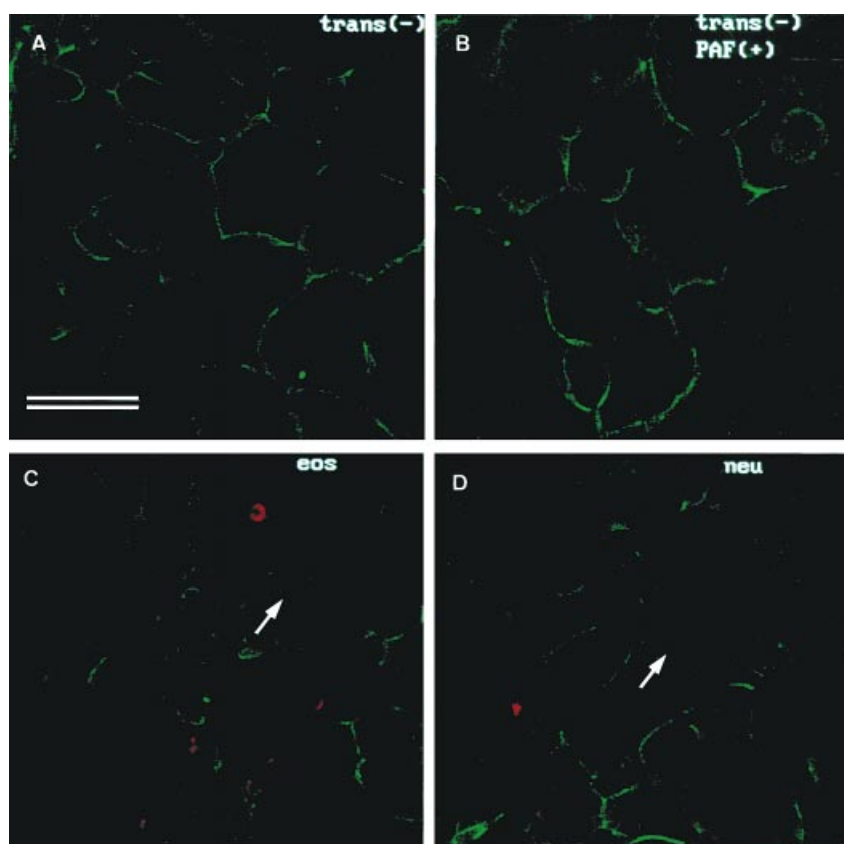


Fig. 3. E-cadherin expression in HNECs after the transmigration assay. (A) The control epithelium without the addition of inflammatory cells nor PAF. (B) The control epithelium with addition of PAF in the lower chamber. (C, D) The epithelium after the migration of eosinophils (C) and neutrophils (D), respectively. E-cadherin immunoreactivity is seen continuously along the adjacent epithelial cell borders (A), and is not notably influenced by the addition of PAF itself (B). After the transmigration of all kind of inflammatory cells, on the other hand, the immunoreactivity appears completely weakened throughout the field, and indeed is not detected in some areas (arrows) (C, D). The surfaces of inflammatory cells are marked red with DiI. Scale bar = 50 μ m.

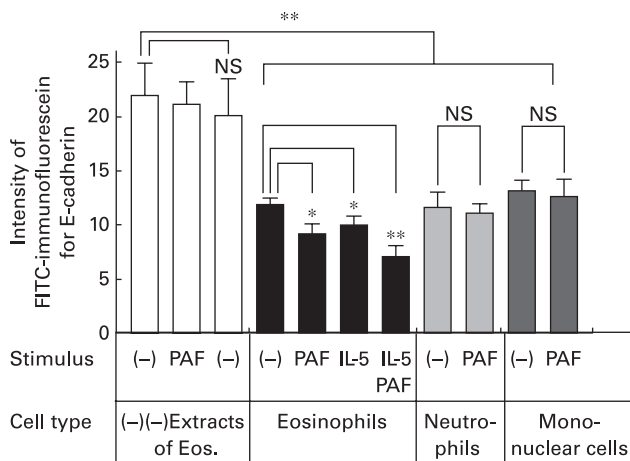


Fig. 4. The mean intensity of immunofluorescence for E-cadherin per square fields of HNECs that have the same area. This mean intensity decreased significantly after the transmigration of all kind of inflammatory cells ($P < 0.01$), without a distinctive difference among cell types in nonstimulated conditions (PAF (-) and IL-5 (-)). In the eosinophil transmigration, the stimulation by either IL-5 or PAF caused significant decrease in the E-cadherin intensity. In addition, more definitive decrease was induced by costimulation with both IL-5 and PAF ($P < 0.01$). In the case of neutrophil transmigration, on the other hand, the mean intensity was unaffected regardless of whether PAF was added or not. Mean $\pm$ SE. * $P < 0.05$, ** $P < 0.01$.

and difference among each cell type was not detectable at light microscopic level.

Figure 4 shows the mean intensity of FITC-immunofluorescence for E-cadherin per square field that have the same area. The means of intensity significantly decreased

after the transmigration ($P \leq 0.01$), and there was no significant difference among the three inflammatory cell types added. In case of eosinophils, a significant decrease of the mean intensity was induced by the stimulation with IL-5 and/or PAF with comparison to the nonstimulated transmigration. As for neutrophils, on the other hand, the mean intensity was unaffected regardless of PAF was added or not.

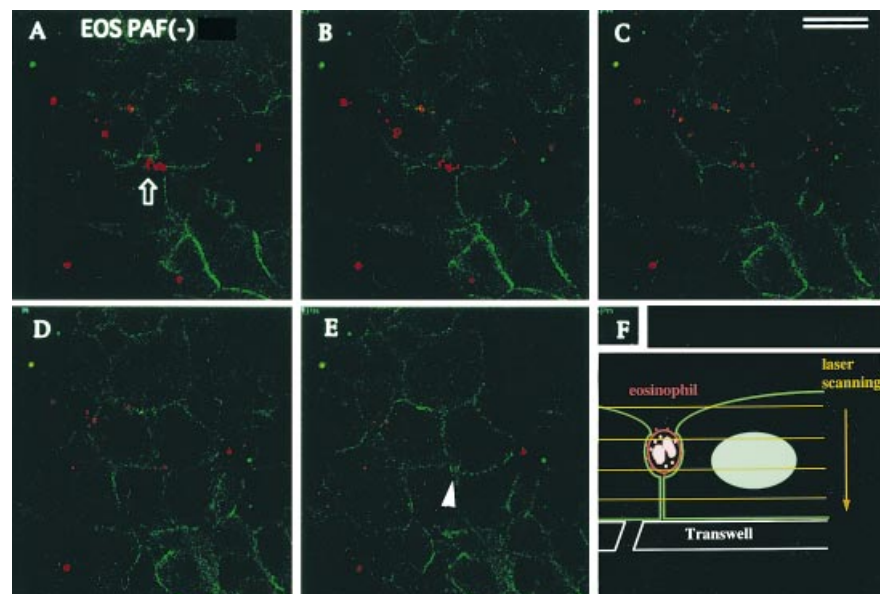
In the control experiments, the addition of PAF alone into the lower compartment without the addition of inflammatory cell, had no notable effect on the E-cadherin immunoreactivity in the HNECs (Fig. 3B, Fig. 4). In case of the supernatants of eosinophil extracts, which were added into the upper compartment in place of inflammatory cells, remarkable changes were observed in neither the histochemical findings nor the mean intensity of E-cadherin immunoreactivity in HNECs (Fig. 4).

Figure 5 shows the immunofluorescein double staining procedures of E-cadherin in HNECs obtained after 1 h assay with eosinophils marked with DiI beforehand. The photographs were taken with gradually changed focus at the same point (Fig. 5F). The E-cadherin immunoreactivity of the HNECs (stained green with FITC) vanished at the points where transmigrating eosinophils (marked red with DiI) interposed between the neighbouring HNECs [arrow]. However, the immunoreactivity remained intact in the different depths where HNECs attached to each other without eosinophil interference [arrowhead].

Discussion

In this study, we demonstrated that the transmigration of

Fig. 5. The immunofluorescein double staining procedure of E-cadherin in the HNECs after 1 hour assay with eosinophils marked with DiI beforehand. Photographs of (A-E) have been taken with gradually changed focus at the same point, as shown in the model (F). The E-cadherin immunoreactivity of the HNECs (stained green with FITC) vanishes at the points where the transmigrating eosinophils (marked red with DiI) interpose between the neighbouring HNECs (arrow). However, the immunoreactivity remains intact in the different depths where the HNECs attach to each other and the eosinophils are not present (arrowhead). Scale bar = 50 μ m.



inflammatory cells through the cultured HNEC monolayers induced a decrease in the epithelial E-cadherin expression. The intensity of the immunofluorescence of the E-cadherin overall decreased in the HNECs after the migration of any kind of inflammatory cells, even in the nonstimulated condition. No significant difference was observed in the degree of decrease induced by transmigration among eosinophils, neutrophils and mononuclear cells. Moreover, the degranulation extracts derived from stimulated eosinophils did not themselves induce significant changes in E-cadherin expression. These results suggest the possibility that the transmigration of any kind of inflammatory cells directly affects the decrease of E-cadherin expression in the HNECs.

The extract derived from eosinophils stimulated both with sIgA and IL-5 also did not cause remarkable changes in E-cadherin expression, whereas the procedure of eosinophil stimulation used in the previous study has been apparently shown to induce eosinophil degranulation [38]. In fact, we measured the concentration of eosinophil-derived neurotoxin (EDN) in the supernatant from incubation of eosinophils and confirmed remarkable increase after the treatment mentioned above (data not shown). IL-5 is well recognized to be a potent chemoattractant of eosinophils in inducing the production of ILs and promoting the release of granule proteins [22–24,38]. Some contents of granule proteins, such as eosinophil peroxidase, eosinophil cationic protein, major basic protein, and some kinds of collagenase, are widely known to have a direct and destructive effect on the airway epithelial cells [10–15], nevertheless our results show that eosinophil extracts themselves do not affect E-cadherin expression in the HNECs. It is therefore suggested that the local decrease in expression of E-cadherin in HNECs may be caused in a different manner, one in which the eosinophil contents, such as granule proteins, are not crucially involved. Although the possibility remains that eosinophil-epithelial cell contact may have resulted in enhanced mediators production and release from eosinophils, which then exert regulatory effects on epithelial E-cadherin.

The direct effect of cell transmigration on the expression of cadherins in tissue-resident cells was previously reported in the relation between polymorphonuclear leucocytes (PMN) and vascular endothelial cells [40–42]. Allport *et al.* have shown the disruption and degradation of vascular endothelial (VE)-cadherin complex in the human vascular endothelium during PMN adhesion and transmigration [40]. In the current work, they revealed that the proteins involved in the VE-cadherin complex are degraded rapidly through a proteolytic mechanism that is dependent on the endothelium and dose not involved granule components of the PMN. Similarly, Maschio *et al.* suggests that PMN-endothelial cell adhesion may be the initiating

signal and that engagement of adhesion molecules may be crucial in triggering intracellular signals in endothelial cell that possibly upregulate VE-cadherin complex disorganization [41]. In the same way, it now seems reasonable to assume that eosinophil-epithelial cell adhesion may induce some intracellular signals in epithelial cells and result in the decrease in E-cadherin expression observed in this study.

It remains unknown why the transmigration of activated eosinophil prominently induces the decrease of epithelial E-cadherin. As compared with other types of inflammatory cells transmigrated, activated eosinophil shows an eminent effect on it. However, this phenomenon is most likely attribute to the remarkable differences in types of adhesion molecules optimal for epithelial cells among eosinophils and the other cells. Nevertheless, we still cannot identify specific ligands that would meet the required criteria, such as expression on eosinophils without expression on neutrophils and mononuclear cells, and binding property to some ligands expressed by epithelial cells.

Several reports were made in recent years concerning the processes in transepithelial migration of eosinophils, in which a certain engagement of adhesion molecules between eosinophil-epithelial adhesion may be involved [26,28]. Bruke-Gaffney *et al.* investigated the role of CD18/ICAM-1 (intercellular adhesion molecule-1) pathway *in vitro*, and suggested that CD18/ICAM-1-mediated eosinophil adhesion to activated human bronchial epithelial cell but that adhesion to resting epithelium was largely independent of this pathway [43]. With regards to the PMN-epithelial cell adhesion, in the same way, Parkos *et al.* suggested that the pair of adhesion molecules involved in an initial adhesive event is quite different from that involved in a subsequent event during the migration of PMN between epithelial cell [44]. They also reported the polarized efficiency in PMN transmigration across a cultured intestinal epithelium; the PMN transmigration in the physiologically relevant (serosal-to-mucosal) direction was 5–20 times more efficient than in the opposite direction (mucosal-to-serosal) [45]. In this study, the transmigration was perfumed in a conventionally established method in which inflammatory cells migrated in the latter direction (mucosal-to-serosal). Although we have not studied the change induced by the migration in serosal-to-mucosal direction, it seems to be possible that transmigration of inflammatory cells in the relevant direction has more effect on the decrease of epithelial E-cadherin expression.

The recent paper indicates that E-cadherin binds heterophilically to a family of integrin molecule [46] and therefore the possibility exists that E-cadherin may play an important role in the eosinophil-epithelial interaction during migration process. Moreover, it was suggested the adhesion and transmigration could induce modifications in the

architecture of epithelial cell-to-cell junctions and facilitates the migration [6,25,42,43]. It is well recognized that E-cadherin is a potent cell adhesion molecule indispensable in the maintenance of the structural and functional rigidity of the epithelium. The blockage of cadherins by antibodies induced tissue destruction and dissociation of the cultured tissue [29] and the expression and localization of E-cadherin was changed by radiation-induced tissue injury [47]. In the previous report, we showed the intimate positional relationship between infiltrating eosinophils, epithelial damage and the loss of the epithelial E-cadherin immunoreactivity by immunoelectron microscopy *in vivo* [25]. This relationship was further evidenced in this study by the observation in the double-staining procedure of E-cadherin of cultured HNEC monolayers and transmigrating eosinophils. Namely, immunoreactivity of E-cadherin was lost specifically at regions of eosinophil adhesion. Furthermore, the strength of the immunoreactivity was semiquantified and shown to be more reduced after the transmigration of the eosinophils activated by both IL-5 and PAF. The reducible effect on E-cadherin was induced by addition of PAF into the medium, which might attribute to the increase in the number of transmigration cells. Although the degree of loss of E-cadherin induced by PAF was quite different between migration of neutrophils and eosinophils. In case of the eosinophils pretreated with IL-5, moreover, E-cadherin decreased significantly in comparison with the case of them pretreated with PBS, whereas the number of transmigration cells has no significant change. From these, we emphasize that the loss of E-cadherin expression is influenced not only by the cell number but also by the activated condition of each eosinophil.

It is well known that IL-5 and PAF are the representative chemoattractant with a wide range of biochemical activities in allergic inflammation [19–24]. The previous reports showed that the pretreatment of IL-5 enhanced the chemotactic effects on eosinophils induced by PAF [24,48]. Practically, histological findings in allergic inflammation showed the predominant infiltration of eosinophils with the highly activated morphology [1–4,9,49]. Considering above, we believe that the result of prominent effect of the activated eosinophils on the decrease of epithelial E-cadherin immunoreactivity has quite importance in the light of epithelial damage in allergic inflammation.

In conclusion, the results of this study demonstrate that the intimate epithelial cell contact mediated by E-cadherin is loosened as a consequence of the transepithelial migration of inflammatory cells, especially activated eosinophils. Such changes in the distribution of cell adhesion molecules in the respiratory epithelium could trigger the damage of epithelial barrier, contributing to the increase in mucosal permeability and facilitating the passage of eosinophils through the epithelium in allergic disorders [16,49,50].

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