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Immunohistochemical Study of E-cadherin and ZO-1 in Allergic Nasal Epithelium of the Guinea Pig

Abstract

Nasal epithelial damage during allergic inflammation was studied by observing the distribution of cell adhesion molecule E-cadherin and tight junction (*zonula occludens*) cell-cell contact associated protein ZO-1. The guinea pig model of nasal allergy, sensitized with intraperitoneally administered ovalbumin (OA) and subsequently challenged with OA intranasally, was used. In control epithelium, E-cadherin immunoreactivity was detected continuously along neighboring epithelial cell borders. ZO-1 spot-like immunoreactivity was detected in the apico-lateral portion of epithelial cells corresponding to the tight junction (TJ) position, but no changes in immunoreactivity were found between control and challenged epithelia. In the challenged epithelium of sensitized animals, marked infiltration of eosinophils and structural changes, such as widening of the intercellular spaces and detachment of adjacent epithelial cells, were observed concurrently. In addition, spots negative for E-cadherin immunoreactivity were noted in the epithelium, associated with the extracellular deposition of eosinophil granule proteins. Immunoelectron microscopy revealed a decrease or disappearance of E-cadherin immunoreactivity, which took place not only in regions where intercellular spaces were wide and adjacent epithelial cells were detached, but also at the point of contact between infiltrating eosinophils and epithelial cells. Approximately 87% of eosinophils observed in the challenged epithelium were associated with such loss of E-cadherin immunoreactivity. These results suggest that the intimate epithelial cell contact mediated by E-cadherin is loosened as a consequence of eosinophil infiltration, which may trigger the initial step of subsequent epithelial destruction in allergic states.

Key Words

Nasal allergy
E-cadherin
ZO-1
Eosinophil infiltration
Epithelial damage
Tight junction
Nasal mucosa

Introduction

Infiltration of eosinophils in the airway mucosa is one of the characteristic features of nasal allergy [1–3] and of bronchial asthma [4–5]. Recent studies have clarified the mechanisms by which eosinophils migrate from capillaries into tissues and pass through the epithelium [6]. This migration process involves both an adhesion process and a directed locomotion under the influence of chemotactic medi-

ators. The interaction of cell adhesion molecules expressed by eosinophils and by tissue resident cells plays a crucial role [6–8].

Another pathological feature of allergic inflammation is the destruction of epithelium, such as epithelial cell shedding and widening of the intercellular space [4, 5, 9]. Several studies have reported that the degree of eosinophil infiltration correlates with the extent of epithelial damage, and results in the development of bronchial hyperreactivity

[9–11]. Filley et al. [12] demonstrated the deposition of major basic protein (MBP) on the damaged bronchial epithelium of a patient who had died of asthma. Further, some eosinophil products such as granule proteins; MBP [13–15], eosinophil peroxidase (EPO), eosinophil cationic protein (ECP) [14, 16], and eosinophil-derived metalloproteinase [17] have been shown to have a great potential to injure the airway epithelium in vitro [14, 18], so eosinophils are considered to be major contributors to allergic inflammation [6, 10, 13, 16].

The respiratory epithelium depends on various adhesion mechanisms in order to maintain its barrier function and epithelial structure [8, 19]. On the lateral border of epithelial cells, the tight junction (TJ = *zonula occludens*; ZO), the adherens junction and the desmosome are among the structures which characterize the intercellular junctional complex of bronchial and nasal epithelium [20]. These cell contacts are mediated by several kinds of cell adhesion molecules, such as cadherins, integrins, the members of the immunoglobulin superfamily, and cell-cell contact proteins including the associated proteins occludin [21], ZO-1 [22], desmoglein and desmocollin [8, 19, 20]. E-cadherin (also called uvomorulin) is one of the best-characterized transmembrane glycoproteins (molecular weight 120 kD) expressed on many types of epithelial cell surface including nasal epithelium [20, 23, 24]. It is involved both in non-junctional and in junctional adhesions [20], usually concentrated in an *adhesion belt* or adherens junction [19, 20, 23, 25], and considered to be the primary mediator of the Ca^{2+} -dependent adhesion between adjacent epithelial cells [8, 20, 23]. TJs are considered to provide a structural paracellular barrier at the apicolateral portion of the epithelium [20, 26, 27]. ZO-1, which is one of the cytoplasmic TJ associated proteins (molecular weight 225 kD [22]), is thought to make contact directly with occludin and anchor it to the underlying cytoskeleton [20, 21, 27].

Cell adhesion mechanisms are expected to show pathological changes which bring about epithelial damage in allergic inflammation. However, little is known about the effects of allergic inflammation on cell adhesion molecules expressed by epithelial cells. In the present study, we have studied the distribution of E-cadherin, as a representative cell adhesion molecule, and ZO-1, as a cell-cell contact protein, during allergic inflammation in nasal epithelium, using a guinea pig model of nasal allergy sensitized with ovalbumin (OA).

Changes in epithelial structure were first observed by transmission electron microscopy. Then immunohistochemistry for E-cadherin, ZO-1 and eosinophil granule proteins of MBP and EPO were performed at both electron-mi-

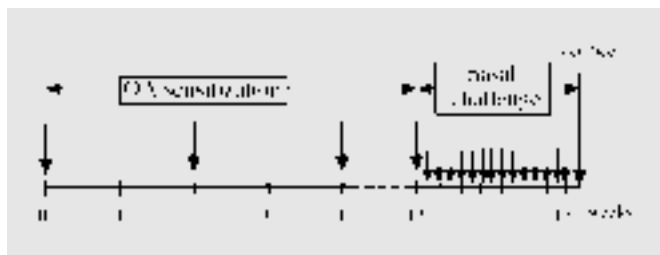


Fig. 1. Schedule of intraperitoneal sensitization and nasal antigen challenge of guinea pigs. Animals were sensitized intraperitoneally with OA and aluminum hydroxide 7 times, once every 2 weeks, followed by unilateral nasal challenge with OA twice a day for 7 days.

croscopic and light-microscopic levels. These observations were statistically analyzed and correlated with eosinophil infiltration.

Materials and Methods

Immunization

Male Hartley guinea pigs, weighing 250–300 g, were first sensitized and then challenged with OA intranasally, as described by Konno et al. [28]. The precise time course is shown in figure 1. Briefly, they were sensitized by intraperitoneal immunization 7 times once every 2 weeks with 20 μg OA as antigen in 1% (w/v) aluminum hydroxide adjuvant in 1 ml of physiological saline. After the last sensitization, the animals were challenged with 20 μl of 1% OA (w/v) in phosphate-buffered saline (pH 7.4), into the left nasal cavity twice a day for 7 days.

In a previous study [28], we showed by passive cutaneous anaphylaxis that, on this immunization schedule, serum anti-OA IgE antibodies and IgG₁ antibodies increased 1,664 $\pm$ 768-fold and 5,632 $\pm$ 2,508-fold, respectively, over 12 weeks.

Tissue Preparations

Twelve hours after the last challenge, animals were perfused through the heart with fixative under deep pentobarbital anesthesia (40 mg/kg body weight). Then the nasal septum mucosa and cartilage were dissected out. According to a previous report [29], we used frontal regions of nasal septum, areas 3 and 4, where the greater part of the mucosa was covered with ciliated epithelia. Five sensitized and 3 control animals were prepared, and samples were collected at least 10 points in these regions in each animal. In each of the following procedures, nasal septum mucosa of three different preparations were compared: (I) antigen-challenged mucosa of sensitized animals, (II) non-challenged mucosa of sensitized animals and (III) mucosa of intact animals, as a control. The challenged and non-challenged mucosa were obtained from the same sensitized animals.

Light Microscopy

Nasal mucosa fixed with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) were embedded in paraffin. They were sectioned frontally at 4 μm , stained with Giem-

sa solution and examined at a magnification of $\times 200$. Eosinophils were identified by both a bilobed nucleus and reddish cytoplasmic granules in the Giemsa stain and counted separately in two zones of nasal mucosa: the epithelial and subepithelial layers. Four areas in each slide were selected randomly for counting, and the number of eosinophils per 1 mm² were calculated.

Differences between preparations were analyzed by unpaired Student's *t* test.

Electron Microscopy

Animals were fixed as described above and the specimens were immersed in fixative overnight. After washing and subsequent postfixation in 1% osmium tetroxide in phosphate buffer (pH 7.4), blockstaining was performed with 1% uranyl acetate in 50% ethanol solution, followed by dehydration through an ascending series of ethanol solutions, passage through propylene oxide, and embedded in Epon 812. Ultrathin sections were stained in uranyl and lead salt solutions.

Immunohistochemistry

Animals fixed with a solution of 4% paraformaldehyde in 0.1 M phosphate-buffered saline through the heart were used in the following study.

For immunofluorescence microscopy, the specimens were immersed overnight at 4°C in 0.02 M Tris buffer saline (TBS) containing 0.01 M CaCl₂ and 20% sucrose, embedded in OCT compound, rapidly frozen, cryosectioned and then mounted on slides coated with APES (3-aminopropyltriethoxysilane). Before application of the primary antibodies, sections were blocked for 1 h with 0.02 M TBS containing 0.01 M CaCl₂, 5% goat serum and 3% bovine serum albumin, and then incubated overnight at 4°C either with rat monoclonal antibody which recognizes the epitope in the extracellular domain of E-cadherin [30] (ECCD-2, Takara, Japan; 1:200) or rabbit polyclonal antibody to ZO-1 (Zymed, San Francisco, Calif., USA, 1:400). Sections were washed in TBS and then incubated for 2 h at room temperature either with FITC-conjugated antibodies to rat IgG or rabbit IgG (Cappel, Organon Technika, Durham, N.C.; 1:200), respectively. For double staining of eosinophil granule proteins, in addition, they were subsequently incubated overnight at 4°C either with mouse monoclonal antibody to MBP (BMK-13, Nichirei, Japan; 1:20) or mouse monoclonal antibody to EPO (Ab-1, Oncogene Science, New York; N.Y., USA; 1:100). The antibody which recognizes guinea pig ECP is not available. After washing, slides were incubated with biotinylated anti-mouse IgG (Vector Laboratories, Burlingame, Calif., USA; 1:200), followed by treatment with Texas red conjugated streptavidin (Vector Laboratories; 1:100) and inspected in a confocal laser scanning microscope (LSM 410, Carl Zeiss, Germany).

For immunoelectron microscopy, 50- μ m-thick sections were prepared using a Vibratome (Oxford Laboratories, USA) and washed in 0.02 M TBS with 0.01 M CaCl₂. After preincubation for 3 h at room temperature in TBS containing 10% normal goat serum, 0.2% bovine serum albumin and 0.1% Triton X-100, sections were incubated overnight at room temperature either with antibody to E-cadherin (1:100) or antibody to ZO-1 (1:200). After rinsing with TBS, sections were incubated for 2 h at room temperature either with biotinylated anti-rat IgG (1:100) or anti-rabbit IgG (Vector Laboratories; 1:100), followed by the avidin-biotin complex (Vector Laboratories) at a dilution of 1:100 for 1 h at room temperature. They were then transferred to 0.05 M Tris-HCl buffer (pH 7.2) and incubated for 10–15 min with 0.02% diaminobenzidine and 1.8×10^{-4} H₂O₂ in 0.05 M Tris-HCl buffer. The sections were fixed overnight with 2.5% glutaraldehyde in

phosphate buffer, postfixed for 1 h in phosphate buffered 1% osmium tetroxide (pH 7.4), blockstained for 1 h with 2% uranyl acetate in 50% ethanol solution, dehydrated and embedded in Spurr's resin. Ultrathin sections were prepared using a microtome and examined in an electron microscope without staining.

Specificity controls were performed both by replacing the first antibodies to E-cadherin or ZO-1 with rat or rabbit IgG proteins (Zymed), respectively, and omitting the first antibodies.

Correlation of E-cadherin Expression with Eosinophil Infiltration

Eosinophil-localized spots were picked up randomly in antigen-challenged epithelium. Epithelial cell membrane facing an eosinophil was assessed for the intensity of E-cadherin immunoreactivity and classified into four grades: grade I, intact expression; grade II, partial decrease in immunoreactivity; grade III, partial disappearance, and grade IV, complete disappearance. At the same time, according to a previous report [9], the epithelial condition of the region was categorized into three groups by the degree of widening of the intercellular space: (I) no widening of the space between epithelial cells; (II) partial widening, and (III) extensive widening. Intercellular spaces free from infiltrating eosinophils were examined in the same manner both in the challenged and non-challenged epithelium. The association between the intensity of E-cadherin immunoreactivity and the degree of epithelial condition was statistically tested by χ^2 test for independence. We also paid attention to the morphology of specific granules in eosinophils, and checked whether they showed the activated pattern, degranulation or not.

Results

Within several minutes after nasal challenge, the animals showed symptoms similar to allergic rhinitis in humans: nasal itching, sneezing, watery nasal discharge and nasal obstruction.

Electron Microscopy

The nasal epithelium in controls showed normal structure of pseudostratified ciliary columnar epithelium (fig. 2a; TJ is shown at a higher magnification). On the challenged side of sensitized animals, eosinophils containing many specific granules were frequently observed in clusters both in epithelial and subepithelial layers (fig. 2b). During the migration from the subepithelial layer into the lumen of the nasal cavity, eosinophils split the basal lamina (fig. 2c) and pass through the intercellular space of epithelial cells and TJ (fig. 2d), subsequently. In the challenged epithelium, the intercellular spaces between epithelial cells were widened, resulting in the detachment of adjacent epithelial cells (fig. 2b).

Tissue Density of Eosinophils

In control animals, few leukocytes were detected in either the epithelial or subepithelial layers of the nasal mu-

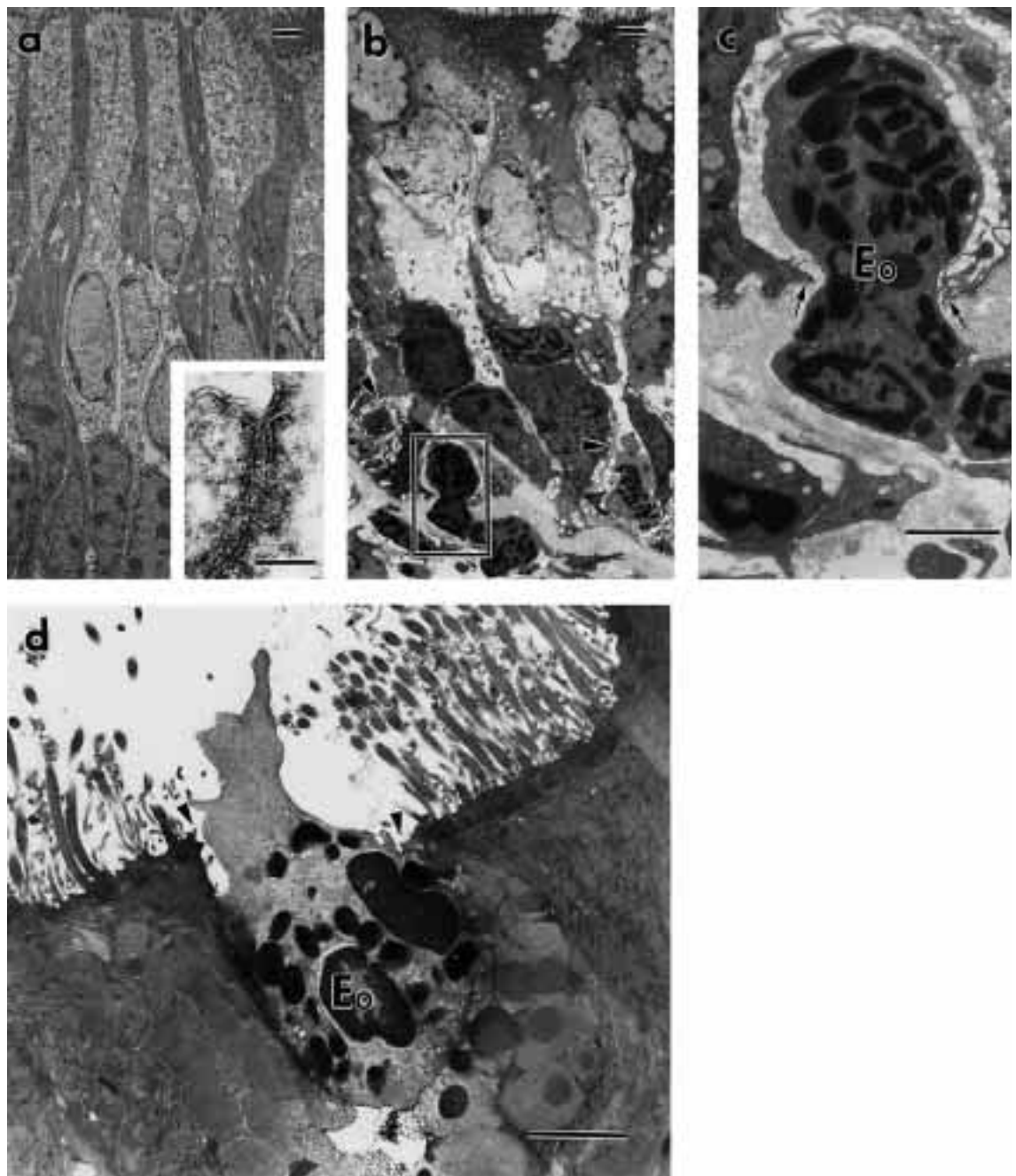


Fig. 2. Electron micrographs of nasal epithelium. **a** Nasal septum mucosa from a control animal showed typical structure of pseudo-stratified ciliary columnar epithelium. Inset, TJ in the apicolateral region. **b-d** Antigen-challenged side of a sensitized animal. Note 'loosened' appearance, widened intercellular spaces (arrowheads) and infiltration of eosinophils (boxed). **c** The boxed area at higher magnification showing an eosinophil (Eo) passing through the basal lamina (arrow). **d** Eosinophil (Eo) passing through the TJ (arrowheads). Scale bars: 2 μ m (**a-d**), 50 nm (**a**, inset).

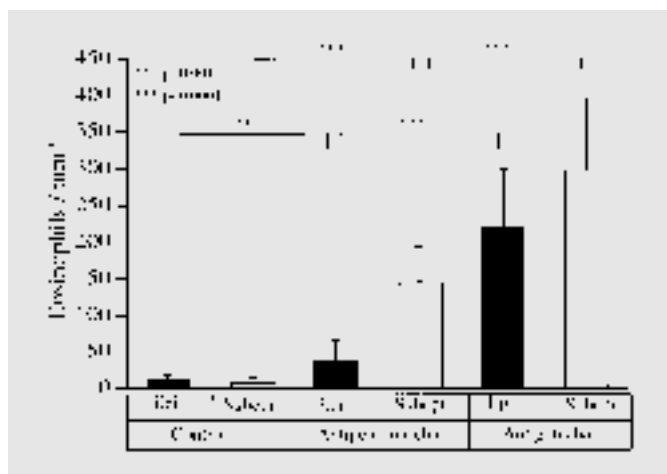


Fig. 3. Tissue density of eosinophils. Each column represents the mean numbers of eosinophils per square millimeter $\pm$ SD. ** $p < 0.001$, *** $p < 0.0001$. Epi = Epithelial layer; Subepi = subepithelial layer.

cosa. In sensitized animals, on the other hand, marked eosinophil infiltration was noted on the challenged side (left nasal cavity), whereas only a few eosinophils were found on the non-challenged side (right nasal cavity).

There were statistically significant differences in the number of eosinophils both in epithelial and subepithelial layers between challenged and control mucosa; non-challenged and control mucosa, and challenged and non-challenged sides of sensitized animals (fig. 3).

Immunohistochemistry

In the specificity control experiments, nonspecific labeling was not detected (data not shown). The immunohistological findings from all samples showed no remarkable differences depending on the location of the samples in each animal.

In control animals, E-cadherin immunoreactivity was seen continuously along neighboring epithelial cell surfaces, most intensely beneath the apicolateral portion (fig. 4a). On the antigen-challenged side (fig. 4c) and non-challenged side (fig. 4b) of sensitized animals, the immunoreactivity appeared weakened in some areas and its distribution showed an irregular pattern. However, these changes in E-cadherin distribution were observed much less frequently on the non-challenged side. Using a double-labeling procedure for E-cadherin and EPO (fig. 5a, b) or E-cadherin and MBP (fig. 5c), colocalization of E-cadherin and those eosinophil granule proteins were detected in the challenged epithelium. The majority of EPO and MBP im-

munoreactivities, which corresponded to contents of the specific granules, concentrated in eosinophil cytoplasm. In addition, spot-like positive staining likely to be released EPO or MBP were observed in the epithelium, where epithelial E-cadherin became negative. In contrast, few eosinophils infiltrated into the non-challenged epithelium, so that staining for EPO and MBP was hardly detected (data not shown).

Immunoelectron microscopy revealed E-cadherin immunoreactivity distributed continuously along the intercellular borders between epithelial cells in control animals (fig. 6a). On the challenged side of sensitized animals, however, E-cadherin immunoreactivity was partially weakened or had disappeared where the adjacent epithelial cells were detached (fig. 6b). Epithelial immunoreactivity for E-cadherin also disappeared around most infiltrating eosinophils. In contrast, E-cadherin immunoreactivity remained intact in interface between adjoining epithelial cells, even in very close proximity to eosinophils (fig. 6b inset, 6c). Such decreases or disappearance of E-cadherin was rare in control epithelium, and scarcely found on the non-challenged side of sensitized animals, but was commonly observed in epithelial membranes adjacent to eosinophils in the challenged epithelium, regardless of whether or not eosinophil-specific granules showed an activated pattern.

To examine the relationship among eosinophil infiltration, epithelial E-cadherin expression and the degree of epithelial damage, we observed 100 intercellular spaces with or without association of eosinophils, respectively, in the challenged epithelium, using immunoelectron microscopy. The same number of intercellular spaces without eosinophils were picked up in the non-challenged side as control. Epithelial E-cadherin immunoreactivity was classified into grades I–IV, which are shown in fig. 7 and defined as described. The results are summarized in table 1. The wider the space of epithelial cells, i.e. the more severe epithelial damage, the weaker epithelial E-cadherin immunoreactivity (statistically significant). In total, the ratio of loss of E-cadherin associated with eosinophils in the challenged epithelium was 87.0%, which was statistically significantly higher than that without association of eosinophils both in the challenged (30.0%) and non-challenged (8.0%) epithelium.

ZO-1 spot-like immunoreactivity was detected in the apicolateral portions of epithelial cells corresponding to the TJs. No remarkable differences were noted among the three types of tissues at light-microscopic levels (fig. 4d–f). With immunoelectron microscopy, ZO-1 immunoreactivity remained intact even in the challenged epithelium, where infiltrating eosinophils apparently passed through the TJ (fig. 6d).

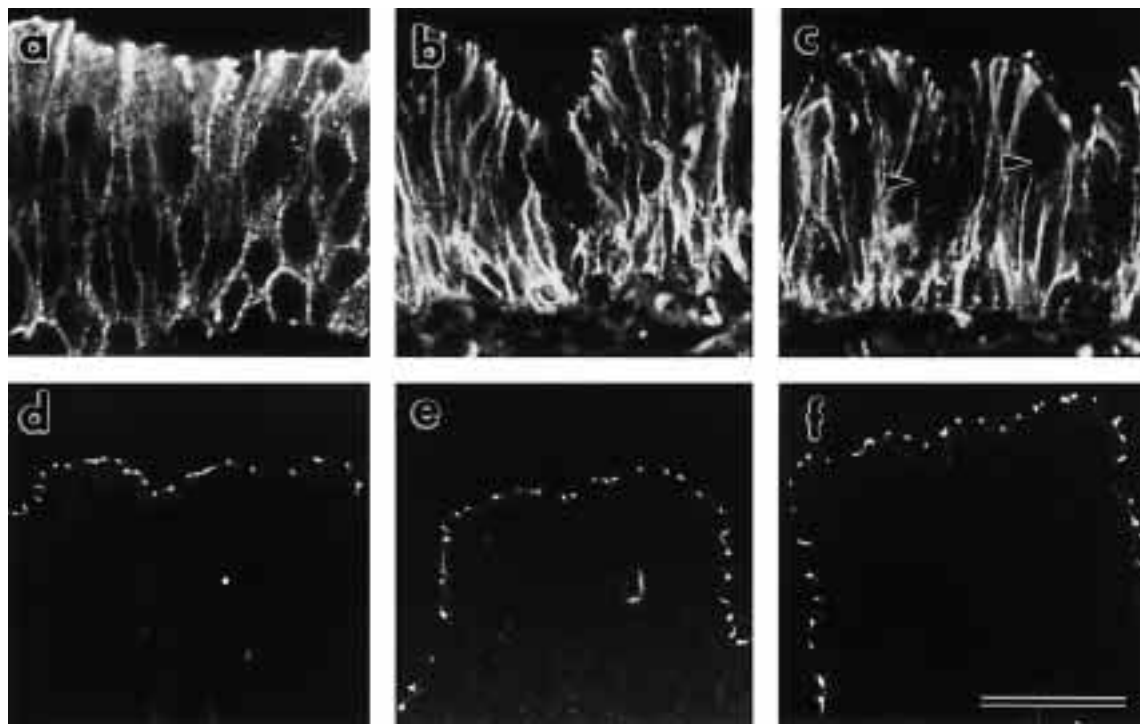


Fig. 4. Immunofluorescent staining for E-cadherin (**a–c**) and ZO-1 (**d–f**) in nasal epithelium of a control animal (**a, d**), non-challenged side of sensitized animal (**b, e**), and antigen-challenged side (**c, f**). In the lateral regions (**c**), patches of E-cadherin-negative areas are visible (arrowheads). No distinct differences were noted among the three ZO-1 samples (**d–f**). Scale bar: 50 μ m.

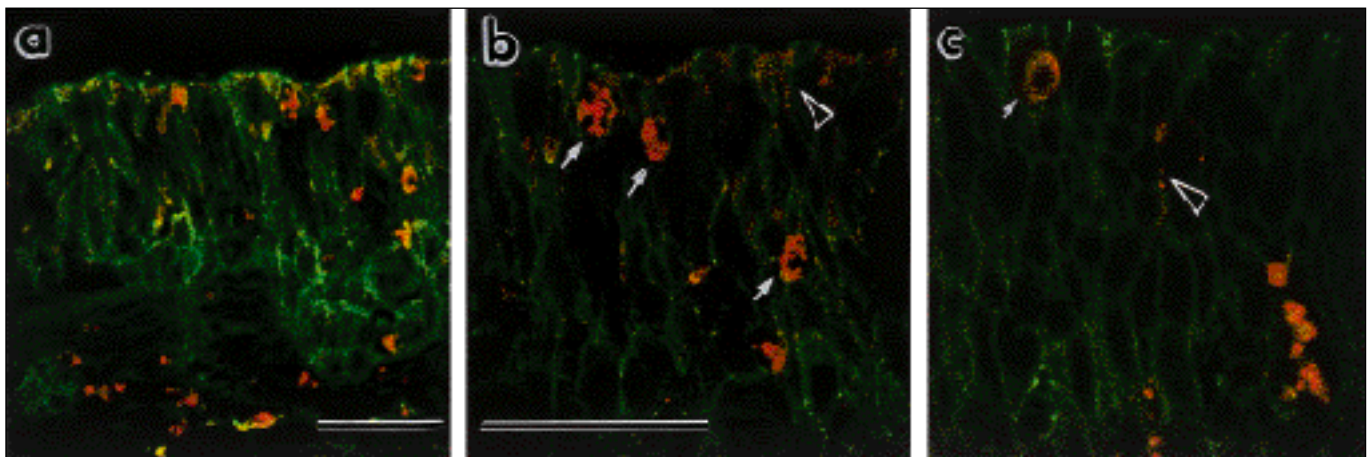


Fig. 5. Double-labeling procedure of E-cadherin (FITC) and EPO (**a, b**) or MBP (**c**; Texas red) in the challenged epithelium. Majority of EPO or MBP immunoreactivity localized in eosinophil cytoplasm (arrows), on the other hand, spot-like positive staining likely to be released EPO or MBP could be pointed by arrowheads, where epithelial E-cadherin could scarcely be detected (arrowheads). Scale bar = 50 μ m.

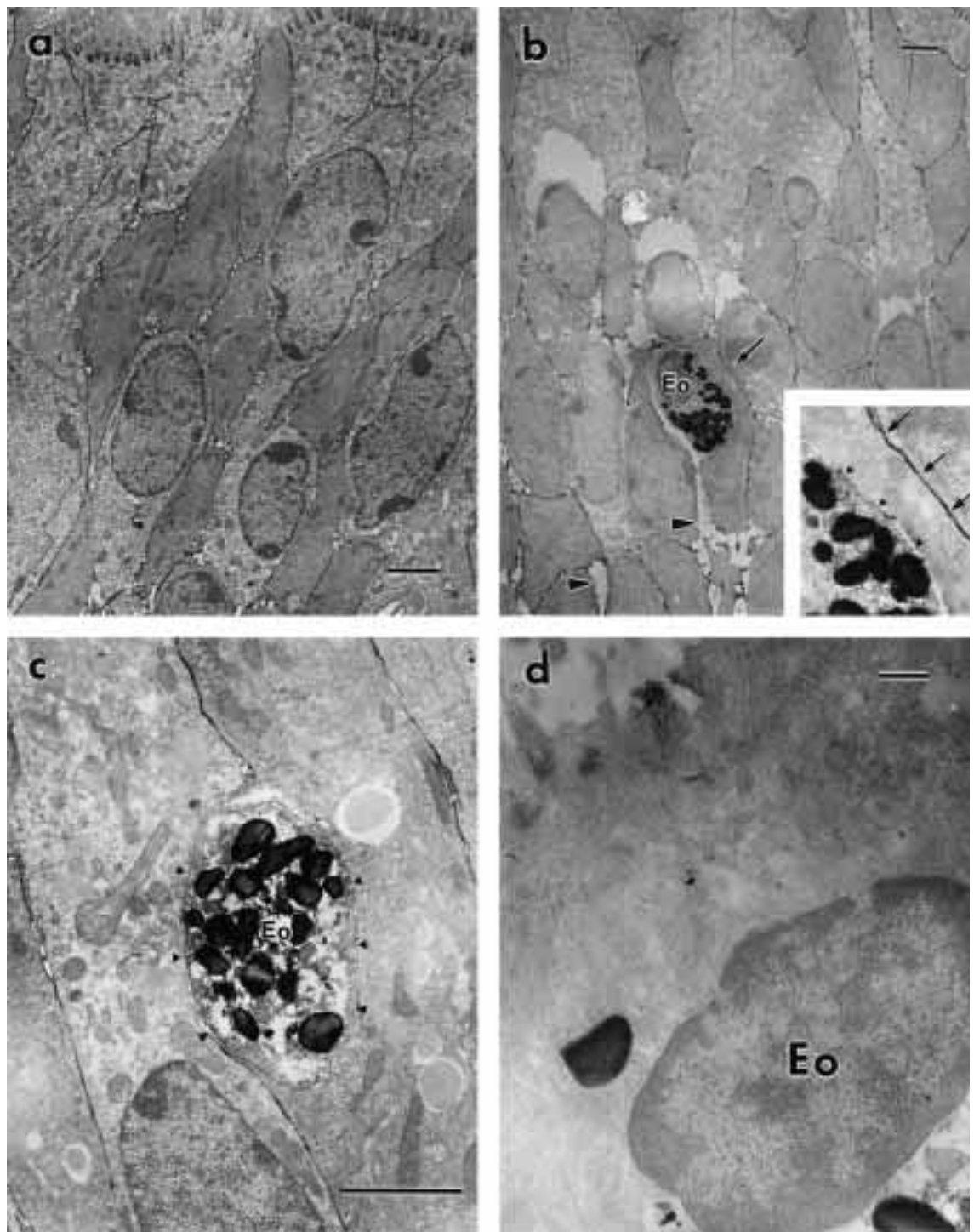


Fig. 6. Immunoelectron microscopy for E-cadherin (**a-c**), and ZO-1 (**d**) in nasal epithelium of a control animal (**a**), and the antigen-challenged side of a sensitized animal (**b-d**). **b** Note detached epithelial cells (arrowheads) and partially weakened E-cadherin immunoreactivity. Inset (arrow) confirms eosinophil-epithelial cell contact is E-cadherin negative (arrowheads), whereas epithelial-epithelial cell interface is E-cadherin positive (arrows). **c** Eosinophil within the challenged epithelium. Again E-cadherin was not detected on the epithelial cell surface in contact with an eosinophil (arrowheads). **d** ZO-1 immunoreactivity in the challenged epithelium, detected apicolaterally (arrow) in the vicinity of an infiltrating eosinophil (Eo). Scale bars = 2 μ m (**a-c**); 500 nm (**d**).

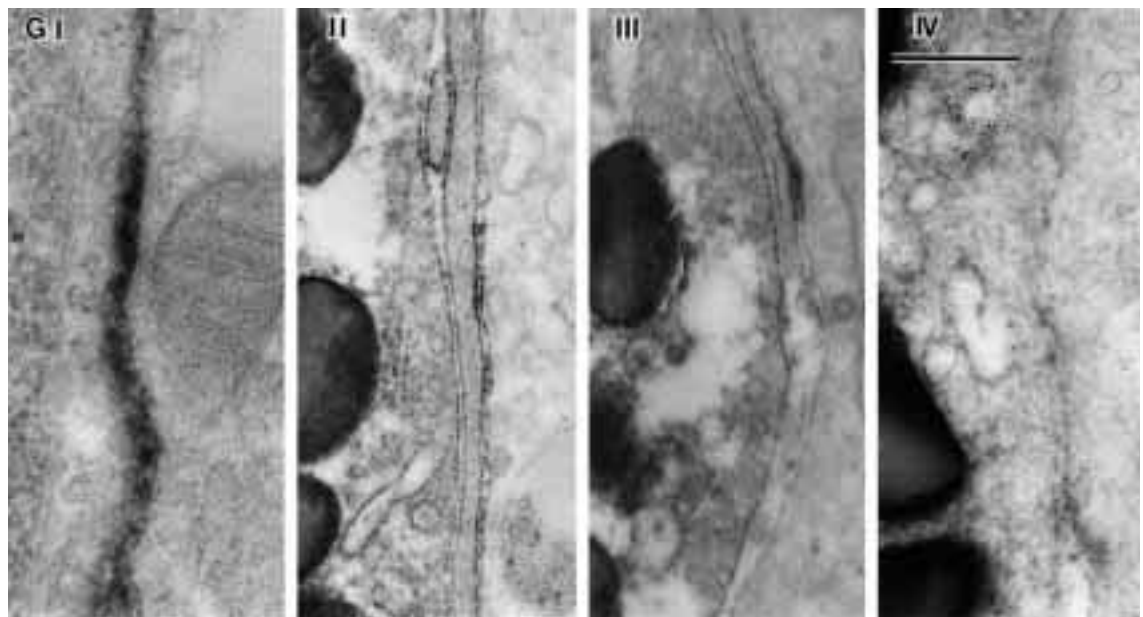


Fig. 7. Electron photomicrographs of the point of contact between epithelial cell and eosinophil (shown in the four degrees as the intensity of E-cadherin immunoreactivity on the epithelial cell membrane. Grade I = Intact expression; grade II = partial decrease in immunoreactivity; grade III = partial disappearance; grade IV = complete disappearance). Scale bar = 500 nm.

Table 1. Loss of E-cadherin at intercellular spaces with or without eosinophil infiltration

Intercellular spaces	Epithelial damage (intercellular spaces)	Total	E-cadherin immunoreactivity ^a				Loss of E-cadherin ^b	
			grade I	grade II	grade III	grade IV	%	n/total n
With association of eosinophils (challenged side)	(I) no widening	32	6	10	10	6	81.3	26/32
	(II) partial widening	26	5	5	11	5	80.8	21/26
	(III) extensive widening	42	2	0	8	32	95.2	40/42
	Total	100	13	15	29	43	87.0	87/100
Without association of eosinophils (challenged side)	(I) no widening	52	47	3	2	0	9.6	5/52
	(II) partial widening	20	15	2	2	1	25.0	5/20
	(III) extensive widening	28	8	4	6	10	71.4	20/28
	Total	100	70	9	10	11	30.0	30/100
Without association of eosinophils (non-challenged side)	(I) no widening	94	90	4	0	0	4.3	4/94
	(II) partial widening	6	2	4	0	0	66.7	4/6
	(III) extensive widening	0	0	0	0	0	0	0/0
	Total	100	92	8	0	0	8.0	8/100

^a E-cadherin immunoreactivity on epithelial cell membrane was classified into four grades as shown in figure 7.

^b Loss of E-cadherin was defined as grades II+III+IV/total.

Discussion

In this study, we report the effects of nasal antigen challenge on the distribution of the cell adhesion molecule E-cadherin and the cell-cell contact protein ZO-1, expressed

by nasal epithelium using a guinea pig model of nasal allergy. As previously reported, the animal model we used showed a positive passive cutaneous anaphylactic reaction with high titer within 12 weeks [28]. This model is similar to human allergic rhinitis both in histological findings and

nasal symptoms induced by nasal antigen challenge [1–3, 28]. We think that the model used in this study has the advantage that the challenged and non-challenged mucosa from the same animal are compared, thus providing an internal control. Marked infiltration of eosinophils was recognized in the challenged mucosa but not in control mucosa. We also observed moderate eosinophil infiltration in the non-challenged mucosa, the mechanism of which is unknown.

In control animals, E-cadherin was found to be distributed continuously along the boundaries between neighboring epithelial cells but most intensively beneath the apicolateral portion corresponding to adherens junction. In contrast, in the challenged epithelium of sensitized animals, E-cadherin immunoreactivity was weakened or undetected in some areas. As seen by immunoelectron microscopy, furthermore, E-cadherin-negative spots corresponded to regions where neighboring epithelial cells were detached, especially around infiltrating eosinophils.

Since the degree of eosinophil infiltration and decrease in E-cadherin expression were most obvious in the challenged epithelium, it was suggested that the local antigen challenge caused these changes, rather than prior systemic sensitization. In addition, our findings by immunoelectron microscopy suggest that eosinophil infiltration, epithelial damage and loss of epithelial E-cadherin immunoreactivity are closely related to each other.

The mechanisms by which eosinophils induce a local decrease in expression of E-cadherin in epithelial cells is presently unclear. Eosinophils are generally recognized as one of the cell types actively involved in allergic inflammation: they are activated during migration from capillaries into the respiratory tract and release granule proteins such as ECP, EPO and MBP, the tissue-toxic effects and the deposition on the asthmatic epithelium of which have been clearly demonstrated [9–18]. In the present study, we observed spot-like positive staining of EPO or MBP associated with epithelial cell negative for E-cadherin. It may therefore be possible that these toxic proteins are involved in decreasing E-cadherin expression by epithelial cells. Otherwise, adhesion of leukocytes to epithelial cells may induce disruption of the E-cadherin complex and loss of its lateral junction localization on epithelial cells. Allport et al. [31] reported the loss of vascular-endothelial cadherin expression in human vascular endothelium during neutrophil adhesion and transmigration, and that was due to the disruption of the VE-cadherin complex and its subsequent degradation, in which an endothelial proteolytic mechanism was involved. It may give us instructive hints to explain the mechanism of changes in E-cadherin expression observed

in this study, whereas the materials and methods are quite different. More definitive experiments *in vitro* are needed to determine whether degradation of E-cadherin in nasal epithelium occurs associating with eosinophil transmigration.

ZO-1 expression was not markedly influenced by antigen challenge in the present experiment, which is in agreement with the previous studies by electron microscopy and freeze-fracture where no significant changes were observed in morphology or in the number of TJ strands in allergic nasal epithelium, in comparison with normal epithelium [32, 33]. ZO-1 is not the TJ protein itself but an associated protein: some other proteins including the transmembrane protein occludin and cytoplasmic associated proteins such as ZO-2 also take part in the formation of the TJ complex [21, 27], so that precise pathological alterations in the TJs remain somewhat unclear. It could be that the TJ complex is more tolerant of damage caused by eosinophil infiltration than the less complex non-junctional adhesion mediated by E-cadherin.

Alterations in E-cadherin expression by nasal epithelium seem to be important from the standpoint of epithelial damage in allergic inflammation, especially since it is known that cell adhesion mediated by cadherins is probably important in maintaining tissue structure [23]. It has also been reported that blockage of cadherins by antibodies induces the tissue destruction and dissociation of cultured epithelial tissue from canine kidney [34]. Kasper et al. [35, 36] reported that expression and localization of E-cadherin changed by following radiation-induced tissue injury in rat and human alveolar epithelium [35, 36]. It is therefore suggested that decreased E-cadherin expression in the nasal epithelium, observed in the present study, could be a trigger for epithelial damage caused by allergic inflammation.

Changes in E-cadherin distribution probably loosen the intimate attachments among epithelial cells, resulting in widening of the intercellular spaces. In intercellular spaces, there are many trigeminal nerve endings which are containing neuropeptides, such as substance P, and those nerves play a role in the development of hyperreactive nasal symptoms in the guinea pig model of nasal allergy [28]. According to Greiff et al., in human airway mucosa, allergic reactions produce mucosal exudation of plasma into airway lumen [37, 38]. Therefore, it is probable that widening of intercellular spaces gives those nerves more chance to be stimulated with chemical mediators released by residual inflammatory cells, leading to hyperreactivity of the nasal mucosa.

In conclusion, the results of this study demonstrate that the intimate epithelial cell contact mediated by E-cadherin is loosened in allergic states as a consequence of eosinophil

infiltration. Such changes in the distribution of cell adhesion molecules in respiratory epithelium may be an initial step which triggers subsequent epithelial destruction, which results in one of the major causes of hyperreactivity in allergic rhinitis.

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