

CD8 and CD103 Are Highly Expressed in Asthmatic Bronchial Intraepithelial Lymphocytes

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Key Words

Intraepithelial lymphocytes • Lamina propria lymphocytes •
Bronchial asthma • CD8 • CD103

Abstract

Background: Although characteristics of intraepithelial lymphocytes (IELs) in mucosal immunity have been well defined in the intestine, bronchial IELs have been little investigated. Recently, we showed that bronchial IELs have a distinct function that partly resembles that of intestinal IELs; however, surface antigen expression of bronchial IELs and the relationship of that expression to airway disease have not been studied. **Methods:** We analyzed phenotypic profiles of human bronchial IELs and lamina propria lymphocytes (LPLs) by double-staining immunohistochemistry using full-thickness bronchial specimens (10 nonasthmatic controls and 7 asthmatics) from lung resections. **Results:** In controls, the percentage of CD4⁺ cells was lower, and the percentage of CD8⁺ cells was higher in IELs compared to LPLs (CD4: median 50.0% in IELs vs. 65.9% in LPLs, $p = 0.01$; CD8: 50.9% in IELs vs. 34.4% in LPLs, $p = 0.007$). The percentage of cells positive for CD103 (α E-integrin) was higher in IELs than that in LPLs (median 60.1% in IELs vs. 16.9% in LPLs; $p < 0.001$). In IELs from asthmatics, these characteristics were particularly

significant (CD4: median 26.2%, $p = 0.008$; CD8: 79.8%, $p = 0.007$; CD103: 76.2%, $p = 0.019$; all compared with IELs from nonasthmatics). **Conclusions:** These results suggest that human bronchial IELs have roles distinct from subsets of other lymphocytes, and that CD8⁺ cells and CD103⁺ cells have potentially important functions in the bronchial epithelium.

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Introduction

Intraepithelial lymphocytes (IELs) reside within the epithelium of systemic mucosal tissues. Among them, intestinal IELs have been confirmed to have phenotypic and functional features different from other lymphocyte populations and to play a crucial role in mucosal immunity in terms of extrathymic differentiation [1], cytotoxicity [2], immunity to infection [3], turnover of epithelial cells [4] and relevance to celiac disease [5]. Their bronchial counterparts, however, have not been well investigated.

T lymphocytes in the respiratory system have been studied by analyzing sputum [6, 7] and bronchoalveolar lavage (BAL) [8]. However, it is not known whether these T lymphocytes originate from the bronchial intraepithe-

lium, subepithelium or lung parenchyma. T lymphocytes have been analyzed immunohistochemically by bronchial biopsy [9, 10], but in these studies only lamina propria lymphocytes (LPLs) were examined because the epithelial layers of small specimens were damaged by the biopsy procedure. It is critical to analyze the characteristics of bronchial IELs, since similar to intestinal IELs they function as the first line of defense against pathogens and likely play important roles in mucosal immunity.

Previously, we investigated the unique characteristics of bronchial IELs by transplanting human bronchial specimens subcutaneously into severe combined immune deficiency mice and found that bronchial IELs in the xenografts survived for more than 6 months, while the numbers of LPLs rapidly decreased [11]. Subsequently, we isolated bronchial IELs and LPLs from resected lung tissue and showed that IELs produced γ -interferon, which stimulated bronchial epithelial cells, and that IELs could be maintained by epithelial cells [12]. However, to date, a comparative evaluation of phenotypes of both bronchial IELs and LPLs in both normal and pathological states has not been made.

In studies of intestinal IELs, investigators have analyzed IELs by conventional separation methods using dithiothreitol, EDTA or proteases [13]. However, such processes can alter the expression of some cellular surface markers [14]. Furthermore, isolation of bronchial IELs using conventional separation methods is challenging and requires short-time incubation of bronchial specimens with interleukin (IL) 2 [12] due to smaller sample size and complex branching structure of the tissue. Such treatment may further alter the cells' surface antigen profile.

Bronchial asthma is characterized by bronchial eosinophilic inflammation, which may be mediated by T lymphocytes, mast cells and other cells. In these types of cells, Th2 type CD4⁺ T lymphocytes may play a central role in allergic airway inflammation [15]. Samples used to study T lymphocytes in asthma have been sputum, BAL or biopsy specimens, and IELs in asthma have not been extensively analyzed.

To define the phenotypic profile of human bronchial IELs and analyze their relationship to airway disease, we undertook double-staining immunohistochemistry of human bronchial specimens from resected lung for cancer and examined IELs and LPLs from both nonasthmatic controls and asthmatics. In addition, to determine whether bronchial IELs differ from those derived from other organs, we compared our data to that generated in previous studies.

Table 1. General characteristics of subjects

	Nonasthmatic controls	Asthmatics
Number	10	7
Age, years		
Median	72.5	67.0
Range	57–79	47–72
Gender (male/female)	6/4	3/4
Smoking (smoker/nonsmoker)	5/5	4/3
Daily treatment	none	ICS 4, T 3, β 5

ICS = Inhaled corticosteroids; T = theophylline; β = inhaled β_2 -agonists.

Materials and Methods

Tissue Preparation

Human bronchial tissue specimens were obtained from 17 patients who underwent lung resection for cancer. Ten were nonasthmatic controls, and 7 were asthmatics who met the American Thoracic Society criteria for asthma [16]. All bronchi from asthmatics showed typical pathological features of asthma: infiltration of EG2⁺ cells, thickening of the basement membrane (BM) and/or hypertrophy of smooth muscle. Subjects did not exhibit symptoms or radiographic findings indicative of other pulmonary disease. Their general characteristics are shown in table 1. Informed consent was obtained from all patients and their families.

Lung specimens were maintained in Krebs-Henseleit solution containing 120 mM NaCl, 4.6 mM KCl, 1.03 mM KH_2PO_4 , 25 mM NaHCO_3 , 1.0 mM CaCl_2 , 1.1 mM MgCl_2 and 11 mM glucose on ice. Lung parenchyma was removed carefully with tweezers, and the third- to fifth-generation bronchi free of tumor invasion were cut into lengths of 5–10 mm, placed in OCT embedding medium (Sakura Finetek USA Inc., Torrance, Calif., USA), rapidly frozen in liquid nitrogen and preserved at -80°C until use. The procedure from resection to freezing required less than 60 min.

All selected specimens were confirmed to show no sign of tumor invasion, inflammation or pathological change other than bronchial asthma, based on microscopic examination

Reagents

The primary monoclonal antibodies used for immunohistochemistry were obtained as indicated in table 2.

Double-Staining Immunohistochemistry

Frozen tissue specimens were sectioned 6 μm thick using a cryostat, placed on glass slides, air-dried for 1 h, fixed in acetone at 4°C for 10 min and air-dried again for 30 min. Circles were drawn around tissue sections using a Dako pen (Dako, Glostrup, Denmark). Endogenous peroxidase was blocked with 0.3% H_2O_2 in PBS, and nonspecific binding was blocked with 5% goat and rabbit serum in PBS. Primary monoclonal antibodies (table 2) appropriately diluted in PBS were applied for 45 min at room tem-

Table 2. Primary antibodies

Specificity	Clone	Composition	Source	Dilution
CD3	UCHT1	mouse (IgG1)	Pharmingen	1:50
CD4	MT310	mouse (IgG1)	Dako	1:100
CD8	DK25	mouse (IgG1)	Dako	1:200
TCR- $\alpha\beta$	8A3	mouse (IgG1)	Endogen	1:50
TCR- $\gamma\delta$	5A6.E9	mouse (IgG1)	Endogen	1:50
CD45RA	L48	mouse (IgG1)	Becton Dickinson	1:50
CD45RO	UCHL1	mouse (IgG2a)	Pharmingen	1:200
CD25	ACT-1	mouse (IgG1)	Dako	1:50
CD103	2G5	mouse (IgG2a)	Beckman Coulter	1:50
CD28	L293	mouse (IgG1)	Becton Dickinson	1:50
CD62L	DREG-56	mouse (IgG1)	Pharmingen	1:50
CD49d	HP2/1	mouse (IgG1)	Beckman Coulter	1:50
CD11a	25,3	mouse (IgG1)	Beckman Coulter	1:50

Source locations: Pharmingen, San Diego, Calif., USA; Dako, Glostrup, Denmark; Endogen, Woburn, Mass., USA; Becton Dickinson, San Jose, Calif., USA; Beckman Coulter, Fullerton, Calif., USA.

perature. Preparations were then washed and incubated with alkaline-phosphatase-conjugated goat anti-mouse immunoglobulin (Dako; 1:100) for 30 min. After blocking with 5% mouse serum, preparations were incubated with FITC-conjugated anti-human CD3 monoclonal antibody (BD Pharmingen, San Diego, Calif., USA; 1:30) for 45 min, washed and then incubated with peroxidase-conjugated rabbit anti-FITC antibody (Dako; 1:100) for 30 min. Enzymatic activity was visualized with a Vector Blue Alkaline Phosphatase Substrate Kit (Vector Laboratories, Burlingame, Calif., USA) according to the manufacturer's instructions. Endogenous alkaline phosphatase was blocked by levamisole. Peroxidase activity was detected with 0.02% 3,3-diaminobenzidine (Dojindo, Kumamoto, Japan) and 0.02% H₂O₂. In short, we immunostained CD3 antigen with brown and target surface molecules with blue; therefore, T cells which did not express target surface molecules were visualized brown, and T cells which expressed target molecules were visualized dull blue (bluish brown) by the mixture of two colors. Counterstaining of nuclei was not performed.

Histological and Immunohistochemical Evaluation of Bronchial IELs and LPLs

IELs were defined as CD3+ cells located within the epithelium. LPLs were defined as CD3+ cells located in the lamina propria, the area between the BM and smooth muscle. The length of the BM and the area of the lamina propria were calculated using NIH image software. IEL number was determined as the number of CD3+ cells per millimeter of the BM, and number of LPLs as the number of CD3+ cells per square millimeter, in both cases using a Vanox AHBS3 microscope (Olympus, Tokyo, Japan). Surface antigen expression was determined by [number of double (brown and blue)-stained cells/(number of double [brown and blue]-stained cells + single [brown]-stained cells)] $\times$ 100, yielding a percentage. More than 200 IELs and 200 LPLs were counted by two individuals blinded to the staining protocol. Results of each count were added and averaged.

Statistical Analysis

Values were expressed as medians and range. Phenotypic comparisons between groups were made using the Mann-Whitney U tests, and χ^2 tests (Fisher's exact tests) were used to compare categorical variables. Correlation coefficients were obtained by Spearman's rank method. $p < 0.05$ was considered statistically significant. All analyses were performed using the SPSS software (SPSS Inc., Chicago, Ill., USA).

Results

General Characteristics of the Subjects

The general characteristics of subjects are shown in table 1. Note that there is no significant difference in age, gender and smoking history between nonasthmatic controls and asthmatics.

Immunohistochemistry of Bronchial IELs and LPLs

Figure 1 shows representative bronchial tissue of non-asthmatic controls. Epithelial layers remained undamaged due to the manner of tissue preparation. CD3+ (T lymphocytes) were stained brown. BMs were identified by microscopic observation, and whether T lymphocytes were located within the epithelial layer (IELs) or within the lamina propria (LPLs) could be easily determined (fig. 1a). Doubly brown- and blue-stained cells were T lymphocytes expressing surface CD4 (fig. 1b), CD8 (fig. 1c) or CD103 antigens (fig. 1d). CD103 expression was apparently dominant in IELs. Table 3 summarizes the number and surface antigen expressions in IELs and

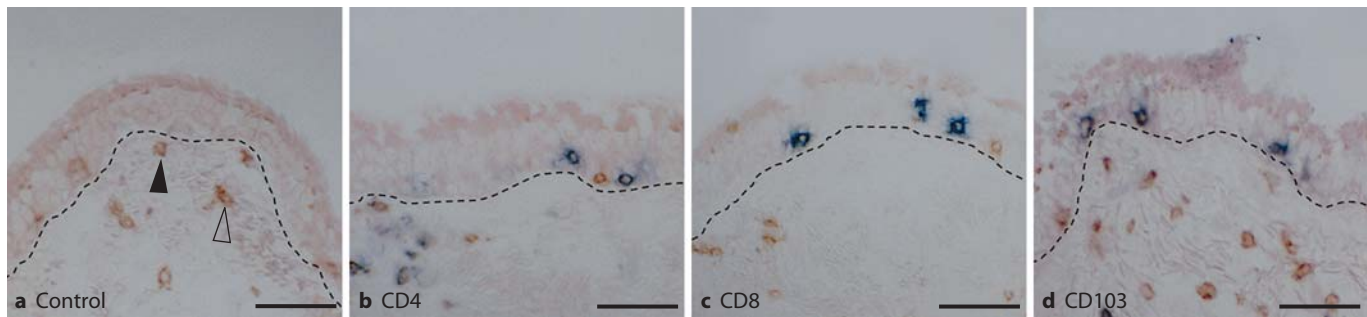


Fig. 1. Representative immunohistochemistry of bronchial tissue in nonasthmatics. CD3 antigen is stained brown, and target surface molecules are stained blue. T lymphocytes applied with control antibody instead of antibodies to targeted surface molecules are stained only brown, not blue (**a**). T lymphocytes expressing CD4 (**b**), CD8 (**c**) or CD103 (**d**) are visualized dull blue (bluish

brown) by the mixture of two colors, and those not expressing target surface molecules are visualized only brown (**b–d**). The black arrowhead in **a** indicates a T lymphocyte which is adjacent to the BM, and the outlined arrowhead in **a** indicates that which is separate from the BM; the dashed line indicates the BM. Bar = 50 μ m.

Table 3. Cell number and surface antigen expression in human bronchial IELs and LPLs

	Controls		Asthmatics	
	IELs	LPLs	IELs	LPLs
Cell number/mm BM	8.3 (7.1–13.7)		10.3 (8.2–28.5)	
Cell number/mm ²		462.5 (323.1–644.0)		532.0 (417.0–834.1) ^b
CD4, %	50.0 (31.2–70.8) ^a	65.9 (46.5–77.9)	26.2 (17.7–51.2) ^{a, b}	59.0 (38.2–73.6)
CD8, %	50.9 (27.3–68.9) ^a	34.4 (18.0–53.8)	79.8 (59.5–87.7) ^{a, b}	47.1 (23.0–69.8)
CD4/CD8 ratio	0.98 (0.46–2.59) ^a	1.85 (0.86–4.23)	0.32 (0.22–0.86) ^{a, b}	1.25 (0.55–3.20)
TCR- $\alpha\beta$, %	96.0 (91.1–100.0)	97.2 (94.6–99.5)	97.8 (83.1–100.0)	97.3 (93.1–98.3)
TCR- $\gamma\delta$, %	2.1 (0.0–6.6)	2.1 (0.0–4.3)	2.9 (0.0–8.6)	1.4 (0.0–7.8)
CD45RA, %	1.1 (0.0–2.6)	2.0 (0.0–7.6)	2.0 (0.0–4.8)	2.9 (1.5–6.7)
CD45RO, %	97.3 (92.6–98.7)	97.3 (93.3–98.7)	94.0 (85.9–95.1) ^b	95.8 (86.9–97.8)
CD25, %	1.8 (0.0–7.4) ^a	6.3 (1.6–18.4)	2.5 (0.0–5.3) ^a	6.2 (2.0–15.5)
CD103, %	60.1 (46.0–71.4) ^a	16.9 (7.5–25.4)	76.2 (47.6–84.5) ^{a, b}	23.0 (17.8–47.6) ^b
CD28, %	41.9 (14.0–62.1) ^a	78.5 (61.4–93.5)	20.8 (11.4–43.5) ^a	82.2 (54.8–92.2)
CD62L, %	0.0 (0.0–1.7) ^a	2.1 (0.0–6.8)	0.0 (0.0–0.8) ^a	2.1 (0.0–3.8)
CD49d, %	81.9 (67.9–93.2) ^a	90.9 (78.0–95.1)	84.7 (78.8–94.1) ^a	95.3 (84.0–98.3)
CD11a, %	98.0 (92.3–100.0) ^a	100.0 (99.1–100.0)	97.4 (92.4–100.0) ^a	100.0 (99.0–100.0)

Data are expressed as medians, with ranges in parentheses. ^a $p < 0.05$ compared with LPLs in the same group of subjects; ^b $p < 0.05$ compared with the same cell population in nonasthmatic controls.

LPLs, and figure 2 shows representative surface antigen expressions in these T lymphocyte populations. Almost no CD19+ cells were observed in the epithelium (data not shown).

IELs and LPLs in Nonasthmatic Controls

The percentage of CD4+ cells was significantly lower, while the percentage of CD8+ cells was higher in IELs

compared to LPLs in nonasthmatic controls (CD4+ cells: median 50.0%, range 31.2–70.8, in IELs vs. 65.9%, range 46.5–77.9, in LPLs; $p = 0.01$; CD8+ cells: median 50.9%, range 27.3–68.9, in IELs vs. 34.4%, range 18.0–53.8, in LPLs; $p = 0.007$; table 3 and fig. 2a, b). The percentage of cells positive for CD103 (α E-integrin) was significantly higher in IELs than in LPLs (median 60.1%, range 46.0–71.4, in IELs vs. 16.9%, range 7.5–25.4, in LPLs; $p < 0.001$;

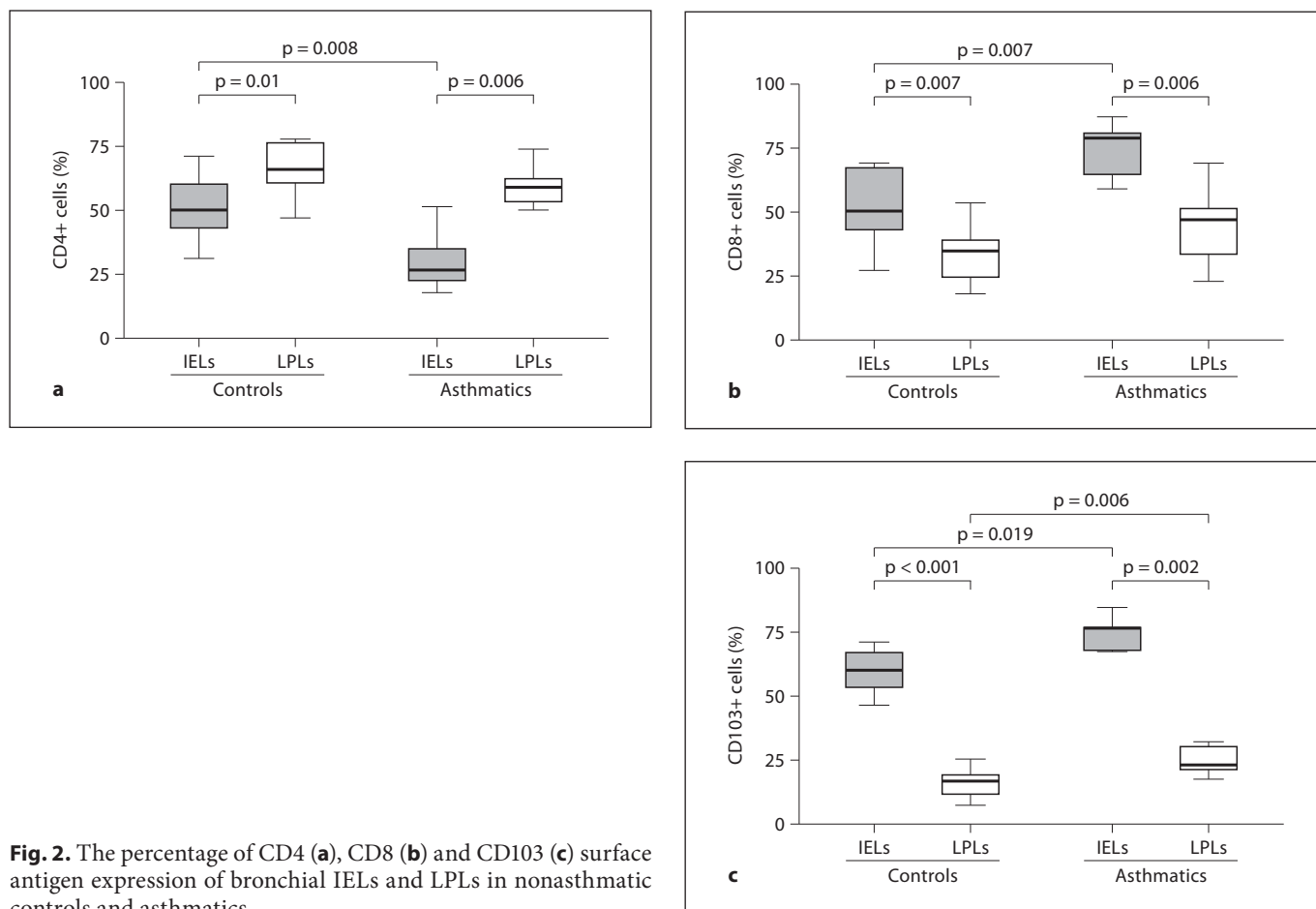


Fig. 2. The percentage of CD4 (a), CD8 (b) and CD103 (c) surface antigen expression of bronchial IELs and LPLs in nonasthmatic controls and asthmatics.

table 3 and fig. 2c). By contrast, the percentage of cells positive for CD25 (IL-2R α), CD28, CD62L (L-selectin), CD49d (α_4 -integrin) and CD11a (LFA-1) was significantly lower in IELs than in LPLs (table 3). Most IELs and LPLs were positive for T cell receptor (TCR) $\alpha\beta$ and CD45RO (table 3).

There was an inverse correlation between the CD4/CD8 ratio and CD103 expression in IELs ($r = -0.73$, $p = 0.02$). As expected, further immunohistochemical staining revealed that CD103 was more highly expressed on CD8+ IELs (median 77.6%, range 73.7–94.1) than on CD4+ IELs (median 35.3%, range 21.7–46.2; $p < 0.001$).

Localization of IELs and LPLs in Nonasthmatic Controls

We next asked whether IELs and LPLs are located adjacent to or separate from the BM in control bronchi. We defined cells as adjacent to the BM when the distance from the center of the cells to the edge of the BM was less

than 8 μm (fig. 1a, black arrowhead); other cells were considered separate from the BM (fig. 1a, outlined arrowhead). Most bronchial IELs were adjacent to the BM in the basal region of the epithelial layer (median 88.2%, range 81.0–97.1), whereas a smaller proportion of LPLs was observed adjacent to the BM (median 15.1%, range 10.6–36.7; $p < 0.001$).

The percentage of CD103+ cells in LPLs adjacent to the BM was higher than that in LPLs separate from the BM (median 21.4%, range 13.4–36.7, vs. 11.9%, range 10.3–19.4; $p = 0.002$).

IELs in Asthmatics and in Nonasthmatic Controls

No significant difference was seen in the numbers of bronchial IELs between nonasthmatic controls and asthmatics (table 3).

Significantly the percentage of CD4+ cells was lower, while the percentage of CD8+ cells was higher in IELs of asthmatics compared to controls (table 3, fig. 2a, b) (CD4:

p = 0.008; CD8: p = 0.007). The percentage of CD103+ IELs was significantly higher in asthmatics compared to controls (table 3, fig. 2c; p = 0.019).

LPLs in Asthmatic and Nonasthmatic Controls

The number of LPLs was greater in asthmatics compared to controls (median 532.0/mm², range 417.0–834.1, in asthmatics vs. 462.5/mm², range 323.1–644.0, in controls; p = 0.048; table 3). In addition, the percentage of CD103+ LPLs was significantly higher in bronchi from asthmatics than in those from nonasthmatics (median 23.0%, range 17.8–47.6, vs. 16.9%, range 7.5–25.4; p = 0.006; table 3, fig. 2c).

Comparison between Smokers and Nonsmokers

In nonasthmatic controls, the number of IELs in smokers compared to nonsmokers (median 8.1/mm BM, range 7.3–8.3, in smokers vs. 8.7/mm BM, range 7.1–13.7, in nonsmokers) was not statistically significantly different (p = 0.07). In asthmatic IELs, CD45RO expression was higher in smokers than in nonsmokers (median 94.9%, range 94.2–95.1, in smokers vs. 93.5%, range 85.9–94.0, in nonsmokers; p = 0.03). Expression of other surface markers shown in table 3 did not differ between smokers and nonsmokers (data not shown).

Differences of IEL Phenotype in Bronchi Compared to Other Organs

Table 4 shows a comparison based on previous published reports of surface antigen expression on IELs from different human organs or tissues. In bronchial IELs, TCR- $\gamma\delta$ expression was lower than that seen in intestinal IELs. The percentage of CD8+ IELs and CD103+ IELs in the bronchus was not as high as that seen in the intestine and nasal mucosa.

Discussion

In this study, we found that CD8 and CD103 were highly expressed in human bronchial IELs compared with LPLs and that these characteristics were particularly significant in the asthmatic bronchi.

The significance of IEL localization close to the BM in bronchi was not unclear also in the intestine. However, we speculate that when the epithelial layer is shed by mechanical injury or inflammation, localization of IELs adjacent to the BM is favorable for them not to be easily lost. In the preparation of bronchial tissues in the present study, we sometimes observed bronchial tissue in which

Table 4. Surface antigen expression of IELs (%) in human intestine, nasal mucosa and bronchus

	Intestine ¹	Nasal mucosa ²	Bronchus ³
CD4	6–12	30	50
CD8	80–90	64	51
CD4/CD8 ratio	0.1–0.2	0.5	1.0
TCR- $\alpha\beta$	60–90	>90	96
TCR- $\gamma\delta$	small intestine: 5–15 colon: 40	<5	2
CD45RA	0	<1	1
CD45RO	90	80–90	97
CD25 (IL-2R α)	little	<1	2
CD103 ($\alpha\epsilon\beta_7$ -integrin)	95	78	60
CD28	32		42
CD62L (L-selectin)			0
CD49d (α_4 -integrin)	80		82
CD11a (LFA-1)	moderate		98

¹ Data from references 17–26.

² Data from Jahnsen et al. [27].

³ Data presented in this study.

epithelial layers were mechanically lost but which kept some IELs attached to the BM. Regeneration of epithelial cells is prompt but that of IELs will not be.

The fact that the percentage of CD8+ cells was higher in bronchial IELs than in LPLs from control subjects suggests that CD8+ IELs could have roles such as defending against foreign pathogenic organisms or influencing the activity of epithelial cells. We have previously shown that many CD8+ IELs produce higher levels of γ -interferon compared with subsets of other lymphocytes in bronchi [12]. Since intestinal CD8+ IELs have a cytotoxic activity toward intestinal epithelial cells [2], bronchial CD8+ IELs may have the same effect on bronchial epithelial cells. In our previous study, the percentage of CD8+ IELs decreased after long-term incubation of human bronchi in the stratum subcutaneum of severe combined immune deficiency mice without stimulation to the bronchial epithelium [11], suggesting that persistent airborne stimuli maintain the number of CD8+ IELs and that their levels decrease in the absence of stimulation. This explanation may also apply to the increased percentage of CD8+ IELs seen in asthmatic airways compared to controls observed in the present study, since epithelial cells in asthma are stressed and damaged [28].

Increased levels of CD8+ IELs in asthmatics suggest that they function in the pathogenesis of asthma. Inter-

Table 5. Studies reporting phenotypes of human bronchial IELs

Authors	Subjects	CD4/CD8 ratio
Fournier et al. [32], 1989	control	0.4
	chronic bronchitis	0.5
Power et al. [33], 1994	control	0.1
Ricciardolo et al. [34], 2003	atopic asthma	0.1
Sjaheim et al. [35], 2006	control	0.4
	occupational asthma	0.4
Tavora et al. [36], 2009	control	1.1
	lung transplant rejection	0.6–0.7

estingly, CD8+ cells in bronchial biopsy specimens of asthma patients express IL-4 and IL-5 mRNAs [29], and bronchial infiltration of CD8+ cells is significantly correlated with decline in lung function associated with asthma [30]. Although previous studies demonstrate that CD4+ cells play central roles in inflammation associated with asthma [31], our studies indicate that CD8+ lymphocytes in mucosa also function in asthma pathogenesis.

In this study we compared the phenotype of human bronchial IELs with data shown in past studies (table 5). In these studies, the CD4/CD8 ratio in IELs of control subjects varied from 0.4 to 1.1, and the expression of CD103 or other molecules was not evaluated. The investigations by Fournier et al. [32], Ricciardolo et al. [34] and Tavora et al. [36] focused only on bronchial IELs and did not examine IELs versus LPLs. Reports by Power et al. [33] and Sjaheim et al. [35] compared phenotypes of IELs and LPLs in control subjects and showed that the CD4/CD8 ratio is lower in IELs than LPLs, similar to our observations. However, the CD4/CD8 ratios reported by both of these authors were lower than what we observed in control subjects. These differences might be due to the fact that tissues evaluated in their study were obtained by transbronchial biopsy of the bronchial carina, whereas our tissues were ring-shaped tubular specimens. While most studies that analyze bronchial specimens in asthmatics focus only on LPLs, two papers shown in table 5 analyzed IELs from asthmatics, and their results differed. Our co-investigation of both bronchial IELs and LPLs on surgically resected tissue in which damage was minimal could provide a means to reconcile discrepancies in comparisons of these T lymphocyte populations.

CD103 preferentially adheres to E-cadherin expressed on epithelial cells of various tissues [37], and human

bronchial epithelial cells also express E-cadherin [12, 38]. These molecules may interact in human bronchial IELs and epithelial cells [12]. The finding in the present study that expression of CD103 was significantly higher in CD8+ IELs than CD4+ IELs indicates that the reason why the percentage of CD8+ cells was higher than that of CD4+ in IELs in particular with asthma may be the interaction with CD103 and E-cadherin. Furthermore, we found a significant correlation between the degree of CD103 expression on T lymphocytes and the proximity of T lymphocytes to the bronchial epithelium. In vitro, TGF- β_1 induces CD103 expression in T lymphocytes [39]. Thus, we hypothesize that either TGF- β_1 released in airway epithelium [40] induces CD103 expression on T lymphocytes or that CD103+ cells preferentially migrate toward bronchial epithelium. Another study indicated that cross-linking of very late activation antigen 4 causes T lymphocytes to express CD103 [41]. Increased levels of TGF- β_1 in BAL fluid from asthmatics [42] and increased deposition of fibronectin (a very late activation antigen 4 ligand) in bronchi from asthmatics [43] may suggest why CD103+ IELs and CD103+ LPLs in asthmatics are increased in our study.

The fact that expression of CD28, CD25, CD62L, CD49d and CD11a was lower in bronchial IELs compared to LPLs and that most IELs express CD45RO indicates that bronchial IELs are a resting memory T lymphocyte population; however, once bronchial IELs are stimulated by CD28, proliferation of lymphocytes increases [12]. We speculate that the reason why the decreased percentage of CD45RO+ IELs in asthmatics compared to nonasthmatics might be that these cells were attracted from the epithelium to bronchial lumen due to an allergic immunoreaction in asthmatics.

A comparison of phenotypes of IELs in 3 human organs (table 4) shows that CD8, TCR- $\gamma\delta$ and CD103 are most highly expressed in the intestine, to a lower degree in the nasal mucosa and least in the bronchus. Unlike the intestine, which has enteric bacterial flora and is exposed to high levels of foreign antigens, the bronchus is practically aseptically and is much less invaded by pathogens, while the nasal mucosa is intermediate between intestine and bronchus. In addition, expression levels of CD8, TCR- $\gamma\delta$ and CD103 are relatively low in circulating lymphocytes. Thus, we hypothesize that these molecules are important in an environment in which lymphocytes often encounter foreign pathogens and epithelial cells are frequently injured.

Our study has some limitations. Our sample size was small, given the difficulty of identifying asthmatic cancer

patients; nonetheless, our results are statistically significant. Second, nonsmokers and smokers are mixed in the study. However, the smokers stopped smoking before lung resection, and there were no statistically significant differences in surface antigen expression between the two populations. Third, some asthmatics used inhaled corticosteroids, which was necessary to prevent asthma exacerbation for the perioperative period. Steroids have been shown to decrease the number of CD4+ lymphocytes but their use does not change the number of CD8+ lymphocytes in bronchi from asthmatics [44]. Also, when we evaluated the number of CD4+ and CD8+ IELs, separately from percentage of expression, it was clear that the number of CD4+ IELs did not decrease but CD8+ IELs did increase in asthmatics compared with nonasthmatic controls. These results suggest that an asthmatic state is associated with increased numbers of CD8+ IELs, rather than with decreased CD4+ IELs, independently of steroid use. Also, all samples came from patients with lung cancer. However, we used bronchial tissues distant from tumors, and histological analysis indicated no pathology except for asthma. Finally, the subjects' age was advanced.

Whereas one study shows that the percentage of CD4+ lymphocytes in BAL fluid increases as women age [45], no study using biopsy samples has been reported, and here the age of controls and asthmatics was similar.

During embryogenesis, the lung bud arises from an endodermal gut tube, which is also the origin of the intestinal mucosa, and branches into the respiratory mucosa. Different temporal or spatial signals likely give rise to the distinct phenotypic profile of IELs among these organs. While many characteristics of intestinal IELs have been intensively investigated in previous studies [46], the role of bronchial IELs in airway immunity merits future analysis.

Acknowledgements

For their kind cooperation in providing human lung tissues, we thank Dr. Yoshioka of the First Department of Surgery, Kumamoto University School of Medicine, Drs. Fujino and Saishoji of Kumamoto Chuoh Hospital, and Dr. Baba of Kumamoto City Hospital, as well as the entire staff of our department.

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