

A Quantitative Analysis of Cedar Pollen-Specific Immunoglobulins in Nasal Lavage Supported the Local Production of Specific IgE, Not of Specific IgG

Tomoaki Yoshida^{*1}, Ayako Usui^{2,*}, Taeko Kusumi², Shigeru Inafuku², Tsuyoshi Sugiyama¹, Naoki Koide¹, and Takashi Yokochi¹

Departments of ¹Microbiology and Immunology, and ²Otolaryngology, Aichi Medical University School of Medicine, Aichi 480–1195, Japan

Received December 20, 2004; in revised form, February 21, 2005. Accepted March 17, 2005

Abstract: Many studies have proved the relevance of local immune responses, rather than systemic immunity, to the pathogenesis of allergic rhinitis. Indeed, allergen-specific B lymphocyte undergoes class switching to IgE *in situ*. However, the relative contribution of *in situ* production to the amount in serum is still ambiguous. Here, a quantitative comparison of the local concentration of allergen-specific IgE with the systemic concentration was explored for the estimation. Among seasonal rhinitis patients, total and Japanese cedar pollen (JCP)-specific IgE, IgA and IgG antibodies were quantified in nasal lavage fluid (NLF) and serum with the time-resolved fluorescence immunosorbent assay. Although the total amounts of IgE and IgG classes in the NLF, which were apparently passive discharge from the mucosal tissue, were smaller and variable, the relative proportions of JCP-specific antibodies could be quantitatively compared between NLF and serum or between subjects. The proportions of specific IgE in the NLF were remarkably higher than in serum (average 13.2-fold) in most subjects, which strongly supported the predominant *in situ* production of the specific IgE and subsequent dilutions in the systemic circulations. Similar but smaller values were obtained for IgA (average 3.7-fold). In contrast, the specific proportions of IgG in the NLF were surprisingly consistent with serum (average 1.0-fold), suggesting that the specific IgG was mostly produced in the downstream lymphoid organs. The local productions of specific IgE would encourage the topical therapies and the usage of the NLF for the diagnosis of allergic rhinitis.

Key words: Allergic rhinitis, Local IgE, Japanese cedar pollen, Time-resolved fluorescence immunosorbent assay

Concerning the pathogenesis of allergic rhinitis, the significance of the local immune response in mucosal tissue, rather than the systemic immune response, has been highlighted for years (3, 9, 13, 19). The provocation test data and clinical symptoms of rhinitis correlated much better with the local level of specific IgE than with serum concentrations (3, 13, 14, 17). Similar phenomena were observed in other allergic diseases, such as conjunctivitis (8) or bronchial asthma (21). In agreement with these observations, several recent studies indicate that IgE class antibodies, either antigen specific or not, are produced *in situ*. First, this is proved by the presence of IgE producing B cells in mucosa, through

the detection of IgE protein (11, 24) or the Cε transcript (4, 6, 22). Second, *ex vivo* cultures of mucosal tissues from patients resulted in the synthesis of antigen-specific IgE protein (15, 20). Moreover, evidence for somatic hypermutation *in situ*, in addition to class switch recombination to IgE, has been reported (2). It is to be noted that the production of the specific antibody in mucosal tissue was predominated by IgE according to the immunohistochemical analysis (11) and *ex vivo* culture (20).

However, the relative importance of the local site in the production of antigen-specific IgE *in vivo* has not yet been comprehensively elucidated, in part due to a limited sensitivity of IgE detection in nasal secretions. Given that the specificity profile of antibodies in the nasal discharge simply reflects the systemic production,

^{*}Address correspondence to Dr. Tomoaki Yoshida, Department of Microbiology and Immunology, Aichi Medical University, 21 Karimata Yazako, Nagakute, Aichi 480–1195, Japan. Fax: +81–561–63–9187. E-mail: Tomo@aichi-med-u.ac.jp

^{*}Present address: Nagoya South Health Co-op Hospital, Nagoya, Aichi 457–0805, Japan.

Abbreviations: JCP, Japanese cedar pollen; NLF, nasal lavage fluid.

the proportions of allergen-specific antibodies in the nasal lavage fluid (NLF) would coincide well with those in serum. On the contrary, if the local concentrations of the allergen-specific antibodies were higher due to *in situ* production, the proportions of such antibodies in the NLF would be higher than in serum. In this study, we introduced the time-resolved fluorescence immunosorbent assay, which enabled about 10–100-fold higher sensitivity than conventional methods. Thus, a quantitative comparison of antigen-specific IgE between local and systemic immunity, namely the NLF and serum, could be performed without any preceding artificial provocation with the antigen.

Materials and Methods

Subjects. Patients of seasonal allergic rhinitis (30 females and 29 males, 7 to 56 years of age with a mean of 33.7) were recruited during the Japanese cedar pollen season from the Aichi Medical University Hospital. Written informed consents from all participants were obtained. Patients were precluded if they had received topical or systemic glucocorticoid therapy within the preceding 3 months or had suffered from upper respiratory infections within 1 month. The nasal lavage was performed by instilling 5 ml of sterile saline to each nostril according to the previous report (10). The serum was drawn subsequently, and the nasal lavage fluids and sera were separated from cells by a centrifugation at $1,500\times g$ to be stored frozen until analysis. The ethics committee of Aichi Medical University had approved this study.

Time-resolved fluorescence immunosorbent assay of antibodies. The total and the antigen-specific IgE, IgG and IgA were measured by time-resolved fluorescence immunosorbent assay (23). Black plates (Maxisorp) from Nalge Nunc International (Rochester, N.Y., U.S.A.) were coated overnight with Japanese cedar pollen antigen (Cosmobio Co., Tokyo) at 0.1 mg/ml for specific antibody analysis, or with appropriate capturing antibodies for total immunoglobulins. In the case of the total IgE measurement, an anti-IgE monoclonal antibody (Sigma Chemical, St. Louis, Mo., U.S.A., 1,000-fold dilution of ascites) was coated. To assay the total amounts of IgA and IgG, a combination of anti- λ and - κ chain monoclonal antibodies (Chemicon International, Inc., Temecula, Calif., U.S.A., 1,000-fold of ascites) was used. The coated plates were blocked overnight with 1% bovine serum albumin and incubated with samples for 3 hr. To detect each class of antibody, biotinylated polyclonal antibodies against IgE (ϵ chain specific), IgG (γ chain specific) and IgA (α chain specific) from Vector Laboratories (Burlingame, Calif.,

U.S.A.) were used at 0.5 $\mu\text{g/ml}$ in 0.5% Tween PBS. The bound biotinylated antibodies were reacted with the Eu^{3+} -labeled streptavidin (Wallac Oy, Turku, Finland) and analyzed with 1420 ARVO multilabel counter (Wallac Oy). The emission fluorescence at 615 nm during 0.4–0.8 msec after pulse excitations at 340 nm was accumulated for analysis. This delayed start of data accumulation made the background of other fluorescent contaminants negligible.

The standard preparations of immunoglobulin G and A were purchased from Nordic Immunological Laboratories (Tilburg, Netherlands) and Protos Immunoresearch (Burlingame, Calif., U.S.A.), respectively. The data of IgE concentrations were standardized to our in-house standard serum, which had been validated by Falco Biosystems, Ltd. (Nagoya, Japan) to WHO reference (75/502). The concentrations of antigen-specific antibodies were calculated through applying the standard curves for total immunoglobulins of each class. In the case of IgE, the values thus obtained were approximately 0.55-fold of RAST score with UniCAP specific IgE kit (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) at Falco Biosystems, Ltd.

The anti- γ chain and anti- α chain antibodies cross-reacted with IgA and IgG, respectively, at the ratios of less than 10^{-4} . The cross reactivity of anti- ϵ antibody to IgG or IgA was less than 10^{-6} , assuming 1 IU as 1 ng. The detection limits of each class were, 0.005 IU/ml (IgE), 3 ng/ml (IgA) and 0.5 ng/ml (IgG). A linear correlation was typically obtained over 4 orders of magnitudes.

Urea assay and normalization of NLF data. The concentrations of urea were measured by the urease-indophenol method using the kit from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The concentrations of immunoglobulins in NLF were normalized with urea concentrations, assuming that urea diffuses freely from serum to nasal secretions (10).

Statistical analysis. The statistic significance of the differences between the paired data was evaluated by calculating the *t*-values on HALWIN software (Gendai-Sugakusha, Kyoto, Japan).

Results

The amounts of total and Japanese cedar pollen (JCP)-specific antibodies in serum and the NLF were quantified by the time-resolved fluorescence immunosorbent assay and proportions of the specific to the total antibodies were calculated in three immunoglobulin classes. Based on less than 10^{-6} cross reactivity of anti- ϵ chain antibody to IgA ("Materials and Methods"), the large excess of JCP-specific IgA in the NLF did not

result in a significant false signal as specific IgE. Namely, even the highest concentration of specific IgA in the NLF (600 ng/ml) corresponded to less than 0.0006 IU/ml of IgE as a cross detection, which was well below the IgE detection limit (0.005 IU/ml). The possible contribution of pollen-specific IgA to the cross detection by anti-IgG was similarly insignificant.

Since the lavage caused unpredictable dilution of secretions, the absolute concentrations in NLF could not be directly compared with those in serum. To circumvent the dilution, the raw data of total IgE and IgG were normalized with urea concentrations, which were assumed to diffuse freely between tissue and secretions (10). After normalization, the total IgE amounts in the NLF ranged from undetectable to 45.8 (median 1.67) IU/ml, while those in serum were from 6.7 to 1,125 (median 88.6) IU/ml. The median value of IgE in the NLF was 50-fold less than that in serum. The normalized mean concentration of IgG in the NLF was 0.30 mg/ml, which was approximately 140-fold smaller than the serum value (44.2 mg/ml). In contrast, the normalized mean concentration of IgA in the NLF was 1.30 mg/ml, which was about 1/6 that of the serum value. The lower concentrations in the NLF confirmed that IgE and IgG in the NLF were predominantly due to passive leakage from the epithelial lining. Among the subjects, the degree of passive translocation into NLF apparently fluctuated to a large extent (undetectable to 16% for IgE, 0.003% to 5.1% for IgG), so the absolute concentrations of IgE and IgG could not be quantitatively compared. Instead, the relative specific antibody proportions in total IgE and IgG deserved quantitative comparisons in a similar way as a previous study (7, 19). The specific antibody amounts were estimated depending on the standard curves for total immunoglobulins of each class. Namely, we assumed that the detection sensitivity and specificity in our assay were comparable between immunoglobulins against JCP and those against other antigens.

First, the proportions of the JCP-specific IgE to the total IgE were calculated in the serum and the NLF. The data from each subject, except from eight subjects mentioned below, were plotted in Fig. 1. In those subjects excluded, the total IgE in NLF were too low to allow quantitative analysis (<0.005 IU/ml), probably due to lower degrees of translocation. The extents of dilution during lavage were not significantly larger in those subjects (22.0-fold of geometric mean) than in other subjects (15.4-fold), as assessed with the concentrations of urea. Three subjects out of the eight excluded were significant in serum RAST score (0.44, 1.31, 7.00), and all of their NLF samples contained trace but detectable amounts of JCP-specific IgE, despite the

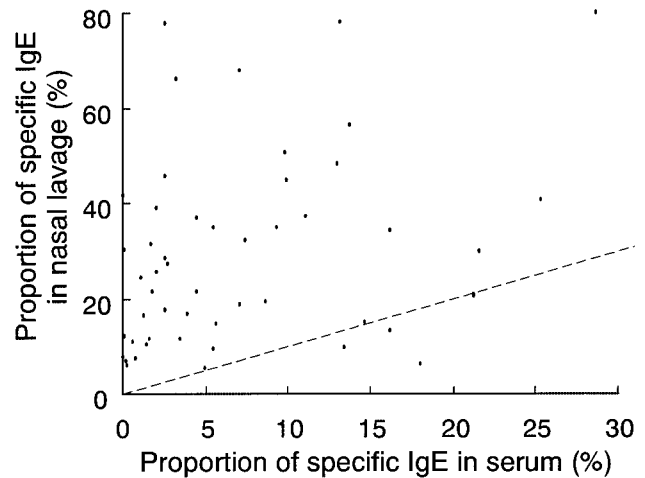


Fig. 1. The proportions of specific to total IgE in the NLF and serum from each subject were plotted. The dotted line indicates the points where the proportions are identical between the NLF and serum.

barely detectable concentrations of total IgE. This observation suggested predominant proportions of IgE in their NLF were specific to JCP, although the reliable values of proportions could not be calculated. In the other five subjects excluded, the serum RAST score were insignificant (0.051–0.262 U/ml), and no specific IgE could be measured in the smaller amounts of total IgE. On the other hand, in four subjects with insignificant but detectable RAST scores in serum (0.011–0.216 U/ml), the JCP-specific IgE could be measured in NLF due to the relatively higher concentration of total IgE, and they were included in Fig. 1.

Among the 51 subjects plotted on Fig. 1, the proportions of specific IgE in the NLF were strikingly higher than those in serum in the majority of cases ($P<0.0001$). The ratio of specific IgE proportions in the NLF versus in the serum ranged from 0.34 to greater than 100, with a geometric mean value of 31.7. It is noteworthy that only four subjects (7.8%) had ratios less than one (ratios=0.34, 0.81, 0.96 and 0.97) and their serum RAST scores were all remarkably high (187.8, 121.2, 14.5 and 27.9, respectively). These differences between NLF and serum indicated that the JCP-specific IgE was predominantly produced *in situ* to constitute the total IgE in the nasal discharge, and a certain portion subsequently merged to the systemic circulation.

When the same comparison was performed with IgA, the proportions of JCP-specific antibodies in the NLF again exceeded those in the serum in many subjects (Fig. 2, $P<0.0001$), indicating the local productions. The mean ratio of specific proportions in the NLF versus the serum was 3.65, which was significantly

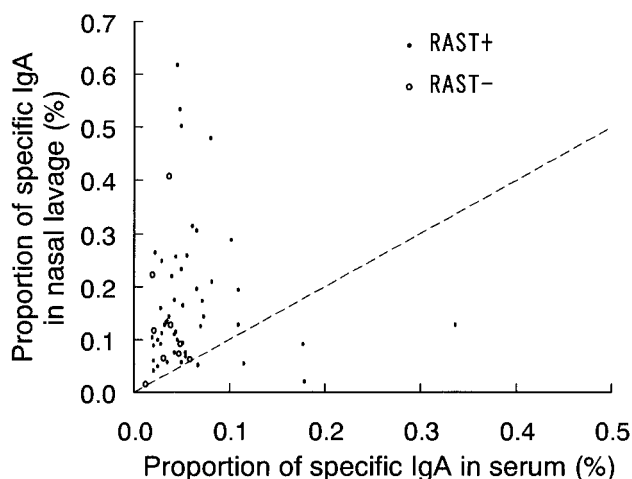


Fig. 2. The proportions of specific to total IgA in the NLF and serum from each subject were plotted. The dotted line indicates the points where the proportions are identical between the NLF and serum.

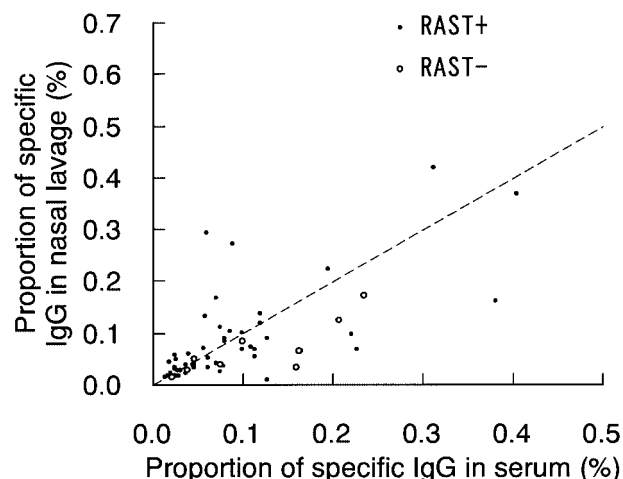


Fig. 3. The proportions of specific to total IgG in the NLF and serum from each subject were plotted. The dotted line indicates the points where the proportions are identical between the NLF and serum.

Table 1. Mean JCP-specific proportions (%) of each immunoglobulin class in the nasal lavage fluid (NLF) and serum

Source	Immunoglobulin classes		
	IgE	IgA	IgG
In the NLF	27.5±17.8	0.196±0.256*	0.078±0.080*
In serum	7.1±6.8	0.065±0.080*	0.089±0.081*
Ratio of NLF/serum	13.2±27.3	3.65±3.03 [†]	1.02±0.77 [‡]

The proportions of specific immunoglobulins were calculated as described in "Materials and Methods" and means±SDs were presented.

Statistically significant by paired *t*-test ($P<0.0001$, *0.005, [†]0.02) in comparison with IgE.

lower than IgE ($P<0.02$). Interestingly, nine subjects with insignificant RAST scores (four included in Fig. 1) also showed similar amounts (data not shown) and proportions of JCP-specific IgA in both NLF and serum (open circles in Fig. 2). The mean specific proportion of IgA in the NLF of those nine RAST negative subjects (0.13%) was not significantly different from that of RAST positive subjects (0.18%).

In contrast, the proportions of JCP-specific IgG in the NLF were highly consistent with the serum data (Fig. 3). The mean ratio of specific IgG proportions between the NLF and serum was 1.02 and was statistically different from the IgE data ($P<0.005$). This mean value strongly suggested that JCP-specific IgG in the NLF derived directly from the systemic circulation. The data of nine RAST negative subjects (Fig. 3, open circles) again did not show significant difference from those of RAST positive subjects.

Next, the proportions of JCP-specific antibody in the NLF and the serum were compared between three immunoglobulin classes. Among the subjects with the

detectable amount of JCP-specific IgE in NLF ($n=51$), the proportions of JCP-specific IgE to the total IgE in the NLF were much higher than IgA or IgG (Table 1). Similar differences were observed in the serum (Table 1).

Discussion

Since IgE and IgG in NLF are not actively secreted through epithelial cells, their concentrations in the NLF were much lower than in serum and fluctuated remarkably. Thus, instead of using the absolute concentrations of IgE or IgG antibodies in NLF, the relative proportions of antigen-specific antibodies to the total immunoglobulin amounts were quantitatively compared between the NLF and serum or between the subjects. Although the antigen-specific antibody amounts were determined assuming that no cross-reactive bindings to JCP were involved in the assay, the assumption would be equally applicable to all three classes of immunoglobulins because the repertoire of antigen binding sites

would be shared among classes. Notably, the values of specific proportions in IgA and IgG observed here were much smaller than a previous report about Cholera toxin antibodies (12), where the affinity-purified polyclonal antibodies were employed as the standards. This fact may suggest that no remarkable cross-reactions to JCP antigen contributed to our data.

The proportions of JCP-specific to the total IgE in the NLF were higher than in serum in most subjects (>92%) who were included in Fig. 1. Although eight subjects could not be included Fig. 1 due to the lower amounts of total IgE in NLF, the presence of specific IgE in NLF was confirmed in three RAST positive subjects among them. These data strongly suggested that IgE was predominantly produced in the local tissues, but not in downstream lymphatic tissues, and subsequently diluted in the general circulations. Interestingly, all subjects with lower specific proportions in the NLF than in serum were with high serum RAST scores. This phenomenon could be rationalized by assuming the involvement of the downstream lymphatic organs due to higher sensitizations. It is noteworthy that JCP-specific IgE could be measured in NLF from four serum RAST negative subjects. This fact might encourage the measurement of the antigen-specific IgE in NLF for clinical diagnosis.

In the case of IgA, to which class switching is enhanced by the presence of lamina propria stromal cells (5), a similar profile of the specific antibody proportions in the NLF and serum was observed (Fig. 2). The results about JCP-specific IgA were in good agreement with a previous report about *Helicobacter pylori*-specific IgA in gastric juice (7). However, the mean ratio of specific proportions in the NLF to in serum was lower in IgA, compared with IgE (Table 1). When taking the active secretions of IgA into account, the higher specific proportions of IgE in the NLF suggested that the extent of *in situ* production of JCP-specific IgE was even larger than IgA. In contrast, specific proportions of IgG were consistent between the NLF and serum (Fig. 3), probably because IgG class was produced in downstream lymphoid organs and recruited to the local sites after recirculation in the blood flow. These results of specific IgE and IgG proportion were in good agreement with a study that mucosal explant from hay fever patients could produce IgE but not IgG antibody, *in vitro* (20). On the other hand, the data about IgG contrasts with a previous report that the specific proportion of IgG is higher in nasal discharge than in serum (19).

The presence of equivalent amounts of JCP-specific IgA and IgG in NLF among serum RAST negative subjects indicates that B lymphocytes are responding to JCP in certain ways but not always switching toward

IgE class. Such JCP-specific IgA and IgG were also found in four asymptomatic volunteers' NLF (our unpublished data). Of great interest to us is the precise mechanism underlining the slants of local immune responses to IgE class switching, which would involve various *in situ* immune cells (1, 4, 18). The presence of JCP-specific IgA in NLF in serum RAST negative subjects may support a possible strategy of inducing a further class switching of B lymphocyte to $\alpha 2$ to ameliorate allergic rhinitis.

The mean proportion of JCP-specific IgE was approximately 100-fold higher than IgA and IgG (Table 1), in both the NLF and serum, and was quite consistent with previous studies with serum (19) or explant culture (20). Such a great difference among immunoglobulin classes could be rationalized by hypothesizing that certain restricted tissues, including the nasal mucosa, are dominantly responsible for total IgE production in modern environments. This idea corresponds with some clinical experiences in which topical steroid therapies resulted in serum IgE decreases (16).

In conclusion, these quantitative analyses of JCP-specific antibodies in the NLF and serum strongly support the local production of antigen-specific IgE antibodies, and not IgG antibodies.

Authors are grateful to Dr. Kazuko Takahashi and Ms. Akiko Morikawa for technical assistance. This work was partly supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

- 1) Cameron, L.A., Durham, S.R., Jacobson, M.R., Masuyama, K., Juliusson, S., Gould, H.J., Lowhagen, O., Minshall, E.M., and Hamid, Q.A. 1998. Expression of IL-4, ϵ RNA, and I ϵ RNA in the nasal mucosa of patients with seasonal rhinitis: effect of topical corticosteroids. *J. Allergy Clin. Immunol.* **101**: 330–336.
- 2) Coker, H.A., Durham, S.R., and Gould, H.J. 2003. Local somatic hypermutation and class switch recombination in the nasal mucosa of allergic rhinitis patients. *J. Immunol.* **171**: 5602–5610.
- 3) Deuschl, H., and Johansson, S.G. 1977. Specific IgE antibodies in nasal secretion from patients with allergic rhinitis and with negative or weakly positive RAST on the serum. *Clin. Allergy* **7**: 195–202.
- 4) Durham, S.R., Gould, H.J., Thienes, C.P., Jacobson, M.R., Masuyama, K., Rak, S., Lowhagen, O., Schotman, E., Cameron, L., and Hamid, Q.A. 1997. Expression of epsilon germ-line gene transcripts and mRNA for the epsilon heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. *Eur. J. Immunol.* **27**: 2899–2906.
- 5) Fagarasan, S., Kinoshita, K., Muramatsu, M., Ikuta, K., and Honjo, T. 2001. *In situ* class switching and differentiation to IgA-producing cells in the gut lamina propria. *Nature* **413**:

- 639–643.
- 6) Fujieda, S., Diaz Sanchez, D., Saxon, A., Kozlowski, P.A., Cu-Uvin, S., Neutra, M.R., and Flanigan, T.P. 1998. Combined nasal challenge with diesel exhaust particles and allergen induces *in vivo* IgE isotype switching. *Am. J. Respir. Cell Mol. Biol.* **19**: 507–512.
- 7) Hayashi, S., Sugiyama, T., Hisano, K., Awakawa, T., Kurokawa, I., Yachi, A., Isogai, H., Isogai, E., Yokota, K., Hirai, Y., Oguma, K., and Fujii, N. 1996. Quantitative detection of secretory immunoglobulin A to *Helicobacter pylori* in gastric juice: antibody capture enzyme-linked immunosorbent assay. *J. Clin. Lab. Anal.* **10**: 74–77.
- 8) Hoffmann-Sommergruber, K., Ferreira, E.D., Ebner, C., Barisani, T., Korninger, L., Kraft, D., Scheiner, O., and Baumgartner, I. 1996. Detection of allergen-specific IgE in tears of grass pollen-allergic patients with allergic rhinoconjunctivitis. *Clin. Exp. Allergy* **26**: 79–87.
- 9) Huggins, K.G., and Brostoff, J. 1975. Local production of specific IgE antibodies in allergic-rhinitis patients with negative skin tests. *Lancet* **2**: 148–150.
- 10) Kato, M., Hattori, T., Kitamura, M., Beppu, R., Yanagita, N., and Nakashima, I. 1995. Soluble ICAM-1 as a regulator of nasal allergic reaction under natural allergen provocation. *Clin. Exp. Allergy* **25**: 744–748.
- 11) KleinJan, A., Vinke, J.G., Severijnen, L.W., and Fokkens, W.J. 2000. Local production and detection of (specific) IgE in nasal B-cells and plasma cells of allergic rhinitis patients. *Eur. Respir. J.* **15**: 491–497.
- 12) Kozlowski, P.A., Cu-Uvin, S., Neutra, M.R., and Flanigan, T.P. 1997. Comparison of the oral, rectal, and vaginal immunization routes for induction of antibodies in rectal and genital tract secretions of women. *Infect. Immun.* **65**: 1387–1394.
- 13) Merrett, T.G., Houri, M., Mayer, A.L., and Merrett, J. 1976. Measurement of specific IgE antibodies in nasal secretion—evidence for local production. *Clin. Allergy* **6**: 69–73.
- 14) Miadonna, A., Leggieri, E., Tedeschi, A., and Zanussi, C. 1983. Clinical significance of specific IgE determination on nasal secretion. *Clin. Allergy* **13**: 155–164.
- 15) Nahm, D.H., and Park, H.S. 1998. Analysis of induced spurtum for studying allergen-specific IgE antibodies in airway secretion from asthmatic patients. *Clin. Exp. Allergy* **28**: 686–693.
- 16) Ohrui, T., Funayama, T., Sekizawa, K., Yamaya, M., and Sasaki, H. 1999. Effects of inhaled beclomethasone dipropionate on serum IgE levels and clinical symptoms in atopic asthma. *Clin. Exp. Allergy* **29**: 357–361.
- 17) Ortolani, C., Miadonna, A., Adami, R., Restuccia, M., and Zanussi, C. 1981. Correlation of the specific IgE in serum and nasal secretions with clinical symptoms in atopics. *Clin. Allergy* **11**: 249–256.
- 18) Pawankar, R., Okuda, M., Yssel, H., Okumura, K., and Ra, C. 1997. Nasal mast cells in perennial allergic rhinitis exhibit increased expression of the Fc epsilonRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. *J. Clin. Invest.* **99**: 1492–1499.
- 19) Platts-Mills, T.A. 1979. Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J. Immunol.* **122**: 2218–2225.
- 20) Smurthwaite, L., Walker, S.N., Wilson, D.R., Birch, D.S., Merrett, T.G., Durham, S.R., and Gould, H.J. 2001. Persistent IgE synthesis in the nasal mucosa of hay fever patients. *Eur. J. Immunol.* **31**: 3422–3431.
- 21) Wilson, D.R., Merrett, T.G., Varga, E.M., Smurthwaite, L., Gould, H.J., Kemp, M., Hooper, J., Till, S.J., and Durham, S.R. 2002. Increases in allergen-specific IgE in BAL after segmental allergen challenge in atopic asthmatics. *Am. J. Respir. Crit. Care Med.* **165**: 22–26.
- 22) Ying, S., Humbert, M., Meng, Q., Pfister, R., Menz, G., Gould, H.J., Kay, A.B., and Durham, S.R. 2001. Local expression of epsilon germline gene transcripts and RNA for the epsilon heavy chain of IgE in the bronchial mucosa in atopic and nonatopic asthma. *J. Allergy Clin. Immunol.* **107**: 686–692.
- 23) Yuan, J., Wang, G., Kimura, H., and Matsumoto, K. 1997. Highly sensitive time-resolved fluoroimmunoassay of human immunoglobulin E by using a new europium fluorescent chelate as a label. *Anal. Biochem.* **254**: 283–287.
- 24) Zurcher, A.W., Derer, T., Lang, A.B., and Stadler, B.M. 1996. Culture and IgE synthesis of nasal B cells. *Int. Arch. Allergy Immunol.* **111**: 77–82.