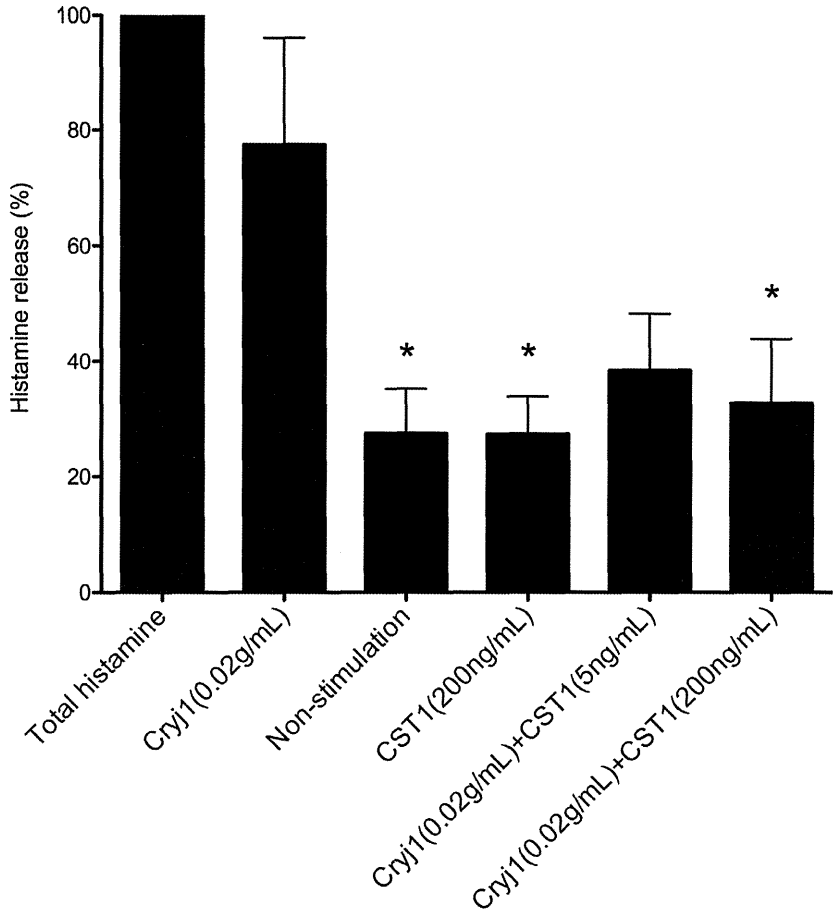


**Figure 4. Histamine release by peripheral basophils stimulated with JC allergen.** Cells were cultured with Cryj1 both in the presence and absence of recombinant CST1. *, $P<0.05$.
doi:10.1371/journal.pone.0067057.g004

support the notion that pathways underlying allergic rhinitis are common regardless of the allergen.

The balance between protease and antiprotease activities might thus be a key to distinguish between the sensitization stage and allergic response, and we suggest that cystatins contributes re-establishing homeostasis of the nasal mucosa.

## Supporting Information

**Figure S1 Immunohistochemical staining of cytokeratin and vimentin in brushed nasal epithelial cells.** Representative immunostaining of cytokeratin expression (A and B) and vimentin expression (C and D). Magnification: ×200.
(PPTX)

**Table S1 Characteristics of subjects for immunohistochemical staining.**
(XLSX)

**Table S2 Manually curated gene sets used for GSEA.**
(XLSX)

## Acknowledgments

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## Author Contributions

Conceived and designed the experiments: Y. Imoto TA EN SF. Performed the experiments: Y. Imoto TT YM YH M. Ono TY Y. Ito. Analyzed the data: Y. Imoto TT EN. Contributed reagents/materials/analysis tools: Y. Imoto TY Y. Ito M. Okano SF. Wrote the paper: Y. Imoto TT TA EN SF.

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ORIGINAL ARTICLE

# Severity Assessment of Japanese Cedar Pollinosis Using the Practical Guideline for the Management of Allergic Rhinitis in Japan and the Allergic Rhinitis and its Impact on Asthma Guideline

Minoru Gotoh[1], Atsushi Yuta[2], Mitsuhiro Okano[3], Nobuo Ohta[4], Atsushi Matsubara[5] and Kimihiro Okubo[1]

## ABSTRACT

**Background:** This study intended to assess the severity of Japanese cedar pollinosis using the Practical Guideline for the Management of Allergic Rhinitis in Japan (PG-MARJ) and the Allergic Rhinitis and its Impact on Asthma (ARIA) Guideline.

**Methods:** An Internet questionnaire survey of patients with pollinosis was conducted in mid-May 2011 and responses were obtained from 3382 individuals who had potential symptoms of Japanese cedar pollinosis from February to early May 2011 and who had experienced such symptoms for at least two pollen seasons.

**Results:** According to PG-MARJ, 23.5% of the respondents had severest rhinitis, 29.4% severe rhinitis, 31.3% moderate rhinitis, 13.8% mild rhinitis and 2.0% asymptomatic rhinitis. According to ARIA, 67.2% of them had moderate/severe persistent rhinitis, 23.8% moderate/severe intermittent rhinitis, 4.4% mild persistent rhinitis and 4.6% mild intermittent rhinitis.

**Conclusions:** Moderate to severe rhinitis was diagnosed in more than 80% of the respondents according to PG-MARJ, while moderate/severe rhinitis was diagnosed in more than 90% of the respondents according to ARIA. Most of the respondents suffered relatively severe pollinosis. More than 80% of the respondents had all the three major symptoms (i.e., sneezing, rhinorrhea and nasal blockage). Disagreement in the severity assessment between the two guidelines was noted in approximately 20% of the respondents.

## KEY WORDS

Allergic Rhinitis and its Impact on Asthma (ARIA), Internet survey, pollinosis, Practical Guideline for the Management of Allergic Rhinitis in Japan (PG-MARJ)

## *INTRODUCTION*

A survey carried out in 2008[1] revealed that the prevalence of perennial allergic rhinitis and seasonal allergic rhinitis (pollinosis) was 23.4% and 29.8%, respectively, and the prevalence of all types of allergic rhinitis was as high as 39.4% in Japan. Seasonal allergic rhinitis in Japan is caused by allergens including pollens of Japanese cedar and cypress in spring, orchardgrass in early summer and ragweed and wormwood in autumn. Among them, Japanese cedar pollen is the most important allergen: the prevalence of

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Japanese cedar pollinosis is as high as 26.5% and the number of patients is increasing year by year. It is assumed that intensive planting of cedar and cypress trees encouraged by the Japanese forestry policy from the late 1950s to the 1960s have resulted in a yearly increase of cedar and cypress pollens dispersed from the grown artificial forest leading to the recent sharp increase of patients with Japanese cedar pollinosis.

Pollens of cedar and cypress start to fall in February every year and their dispersal is observed until early May. This large-scale Internet questionnaire survey was conducted in mid-May 2011 when dispersal of cedar and cypress pollens had almost ended. The subjects of the survey were individuals who had symptoms of Japanese cedar pollinosis from February to early May 2011 and who had experienced such symptoms for at least two pollen seasons.

In Japan, the Practical Guideline for the Management of Allergic Rhinitis in Japan (PG-MARJ)[2] that describes standard diagnosis and treatment strategies has been widely used and the severity of allergic rhinitis has been classified based on the three major symptoms (i.e., sneezing, rhinorrhea and nasal blockage) into five grades (i.e., severest, severe, moderate, mild and asymptomatic). On the other hand, the Allergic Rhinitis and its Impact on Asthma (ARIA) Guideline[3] that is regarded as the international guideline for allergic rhinitis divides the disease into persistent and intermittent types and classifies the severity as mild or moderate/severe depending on the disease's impact on the daily life activities of the patients. When the disease type and the severity are combined, allergic rhinitis can be classified into four categories: moderate/severe persistent, moderate/severe intermittent, mild persistent and mild intermittent.

This survey included questions about the severity of symptoms and the impact on daily life activities; moreover, it intended to assess the severity of Japanese cedar pollinosis according to PG-MARJ and ARIA and examine differences in their assessment.

## *METHODS*

### SURVEY PERIOD

The survey was conducted in the 8-day period from May 11 to May 18, 2011 when the pollen dispersal had almost ended.

### CONTRACTED RESEARCH COMPANY

ANTERIO Inc. (Tokyo, Japan) was contracted to conduct the survey via INTAGE Inc. (Tokyo, Japan), a net monitor (Cue Monitor/Yahoo! Research Monitor).

### SUBJECTS

Net monitors of INTAGE Inc. have been registered after strict proof of identity by sending a registration form to their home address. A broad range of attributes of monitors including their profile were collected at recruitment.

All monitors in the country, except those in Hokkaido and Okinawa where there are very few patients with Japanese cedar pollinosis, were screened to select those who met the following criteria:

(1) Individuals who had potential symptoms of Japanese cedar pollinosis (e.g., sneezing, nasal discharge, nasal obstruction, itchy eyes and teary eyes) in the pollen season from February to May 2011.

(2) Individuals who had experienced those symptoms for at least two pollen seasons.

(3) Individuals who were not healthcare professionals or worked at a publicity agent or market research company, or had any family member, friend or acquaintance who was a healthcare professional or worked at a publicity agent or market research company.

### METHODS

This questionnaire survey was conducted on the Internet. Since registered monitors are individuals aged 15 years or older, responses about patients aged 2 to 14 years were given by their mother or female guardians who were registered as monitors. Specifically, monitors were asked at screening for the survey whether they had a child aged less than 15 years who had potential symptoms of pollinosis from February to May 2011 and who had had the symptoms in 2010 or before. Monitors who had such a child were asked to participate in the survey and answered the questions about their child (only one child even if they had more than one child with pollinosis).

The policy for use of data obtained in the survey and protection of personal information was displayed when the monitors first visited the Web site for the survey and only those that agreed with the policy were allowed to answer the questionnaire.

### QUESTIONS

The questionnaire consisted of questions that were prepared based on classifications in PG-MARJ and ARIA. Responses were used to assess the severity of the condition in each respondent according to PG-MARJ and ARIA separately. Generally speaking, Internet survey may be less reliable because the monitors themselves judge the degree of their illness. To deal with that problem, we made sure that the questions should be clear so that the monitors wouldn't mistake the meaning.

Q1 When did you notice symptoms that could indicate pollinosis (e.g., sneezing, nasal discharge, nasal obstruction, itchy eyes and teary eyes) ? Please mark all that apply.
a) February 2011, b) March 2011, c) April 2011, d) May 2011

**Table 1** Distribution of the respondent population by age

| | <10 years | 10s | 20s | 30s | 40s | 50s | ≥60 years | Total |
|---|---|---|---|---|---|---|---|---|
| Male | 113 | 223 | 237 | 279 | 282 | 243 | 331 | 1708 (50.5%) |
| Female | 128 | 252 | 238 | 277 | 276 | 247 | 256 | 1674 (49.5%) |
| Total | 241 (7.1%) | 475 (14.0%) | 475 (14.0%) | 556 (16.4%) | 558 (16.5%) | 490 (14.5%) | 587 (17.4%) | 3382 |

Q2 When did you visit a medical institution (e.g., hospital, clinic) for symptoms of pollinosis or for the treatment/prevention of pollinosis? Please mark all that apply.
a) May 2011, b) April 2011, c) March 2011, d) February 2011, e) January 2011, f) Not from January to May 2011 but in the past, g) Never

Q3 Did you use any over-the-counter (OTC) drug (sold at a pharmacy or drug store) from February to May 2011 for symptoms of pollinosis?
a) Yes, b) No

Q4 How often did you experience each of the following symptoms in the period with the severest symptoms? Please select only one for each question.
(1) Sneezing (average number of episodes per day)
a) 21 or more, b) 11 to 20, c) 6 to 10, d) 1 to 5, e) None
(2) Nasal discharge (average number of blowing per day)
a) 21 or more, b) 11 to 20, c) 6 to 10, d) 1 to 5, e) None
(3) Nasal obstruction
a) Complete obstruction all day long
b) Very severe nasal obstruction with mouth breathing most of the time
c) Severe nasal obstruction with mouth breathing several times per day
d) Nasal obstruction without mouth breathing
e) No nasal obstruction

Q5 What was the average number of days per week with any symptoms of pollinosis?
a) 4 or more days per week on average, b) Less than 4 days per week on average

Q6 How long did the symptoms of pollinosis persist?
a) 4 consecutive weeks or longer, b) Less than 4 consecutive weeks

Q7 Did you have the following during the period with the severest symptoms?
(1) Sleep disorder
a) Yes, b) No
(2) Disturbance during daily life, leisure and/or sport activities
a) Yes, b) No
(3) Disturbance during school work or work
a) Yes, b) No
(4) Any bothersome symptom
a) Yes, b) No

## *RESULTS*

### DEMOGRAPHIC AND OTHER CHARACTERISTICS OF THE RESPONDENTS

In the Internet questionnaire survey conducted between May 11 and May 18, 2011, responses were obtained from 3382 monitors who had symptoms of pollinosis from February to May 2011 (e.g., sneezing, nasal discharge, nasal obstruction, itchy eyes and teary eyes) and who had experienced the symptoms for at least two pollen seasons. The mean age of the overall population was 38.2 years (range: 2 to 81 years). They comprised 1708 males (mean age: 39.3 years) and 1674 females (mean age: 37.1 years). Table 1 shows the distribution of the respondent population by age.

As for symptoms of pollinosis, 1655 respondents (48.9%) had presented the symptoms in February, 2804 (82.9%) in March, 2822 (83.4%) in April, and 1790 (52.9%) in May 2011 (respondents could have presented the symptoms in more than one period). A total of 1417 respondents (41.9%) visited a medical institution for symptoms of pollinosis or for the treatment/prevention of pollinosis between January and May 2011, while 1133 respondents (33.5%) did not visit any medical institution in that period but had visited one before and 832 respondents (24.6%) had never visited any medical institution. In addition, 1080 respondents (31.9%) used OTC drugs for symptoms of pollinosis

### SEVERITY ACCORDING TO PG-MARJ

Figure 1 shows the severity in the period with the severest symptoms in 3382 respondents: (1) sneezing (average number of episodes per day), (2) nasal discharge (average number of blowing per day) and (3) nasal obstruction (degree). Based on the severity of these three symptoms, the severity of rhinitis in each respondent was assessed using the five-grade classification of PG-MARJ. As a result, 795 respondents (23.5%) had severest rhinitis, 995 (29.4%) severe rhinitis, 1057 (31.3%) moderate rhinitis, 468 (13.8%) mild rhinitis and 67 (2.0%) asymptomatic rhinitis. In addition, the disease type was determined based on the combination of severity of sneezing, rhinorrhea and nasal blockage. The distribution of disease type in the 3382 respondents is shown in Table 2. Actually,

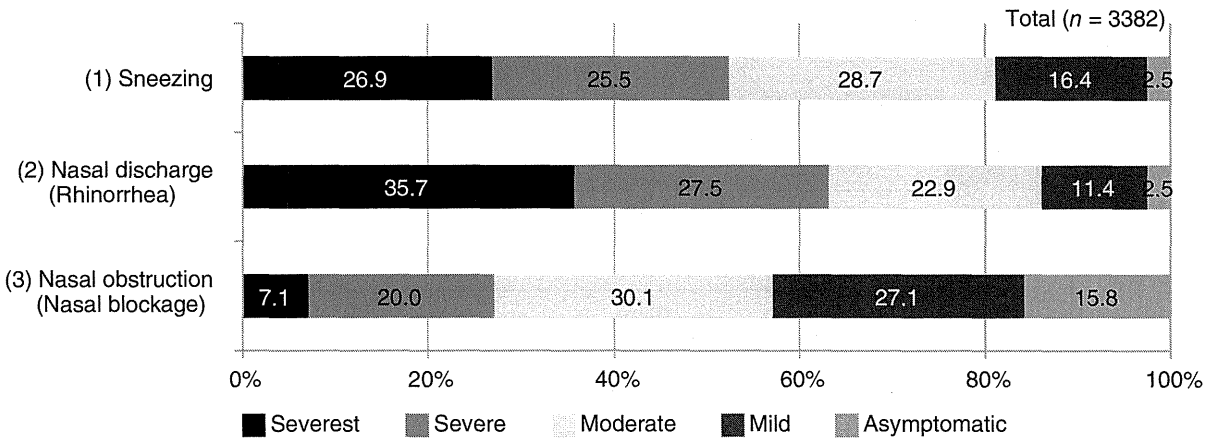


**Fig. 1** Symptoms of pollinosis according to PG-MARJ.

82.0% of the overall population had all three major symptoms (i.e., sneezing, rhinorrhea and nasal blockage). The severity by age group is shown in Figure 2.

## SEVERITY ACCORDING TO ARIA

1) Disease type

In the ARIA Guideline, the term "persistent" denotes presence of symptoms for ≥4 days per week and for ≥4 weeks, while the term "intermittent" denotes presence of symptoms for <4 days per week or for <4 weeks. The average number of days with the symptoms of pollinosis per week was ≥4 days in 2750 respondents (81.3%) and <4 days in 632 respondents (18.7%). The duration of the symptoms was ≥4 weeks in 2675 respondents (79.1%) and <4 weeks in 707 respondents (20.9%).

Based on the above results, 2423 patients (71.6%) had persistent rhinitis (with symptoms for ≥4 days per week and for ≥4 weeks) and 959 patients (28.4%) had intermittent rhinitis.

2) Severity

Figure 3 summarizes the presence of (1) sleep disorder, (2) disturbance during daily life, leisure and/or sport activities, (3) disturbance during school work or work and (4) any bothersome symptom during the period with the severest symptoms of pollinosis. According to ARIA, presence of any of these four conditions [ (1) to (4) ] is regarded as moderate/severe rhinitis, and absence of all of them as mild rhinitis. Based on the replies to these questions, 3079 respondents (91.0%) had moderate/severe rhinitis and 303 respondents (9.0%) had mild rhinitis.

3) When ARIA was applied to the above replies from 3382 respondents, 2274 respondents (67.2%) had moderate/severe persistent rhinitis, 805 (23.8%) moderate/severe intermittent rhinitis, 149 (4.4%) mild persistent rhinitis and 154 (4.6%) mild intermittent rhinitis. The distribution of the overall population by severity and age group is shown in Figure 4.

## PROPORTION OF RESPONDENTS VISITING A MEDICAL INSTITUTION

The proportion of respondents visiting a medical institution was calculated for each degree of severity determined separately according to PG-MARJ and ARIA. In the overall population, 41.9% of the respondents visited a medical institution for symptoms of suspected pollinosis between January and May 2011, while 33.5% did not during that period but had visited a medical institution before. Thus, 75.4% had visited a medical institution regardless of the time and 24.6% had never visited one. As shown in Figure 5, respondents were more likely to have visited a medical institution as the severity level according to PG-MARJ increased. However, approximately 20% of the respondents with severest or severe rhinitis had never visited a medical institution for pollinosis. On the other hand, respondents considered to have persistent rhinitis according to ARIA were more likely to have visited a medical institution than those considered to have intermittent rhinitis, as shown in Figure 6. In the overall population, 31.9% used OTC drugs between February and May 2011. Of the 1417 respondents who visited a medical institution between January and May 2011, 17.3% used OTC drugs in contrast to 48.2% of the 1133 respondents who did not visit any medical institution during the same period but who had visited a medical institution before and 34.7% of the 832 respondents who had never visited any medical institution.

## CORRELATION OF SEVERITY ASSESSMENT BETWEEN PG-MARJ AND ARIA

Figure 7 shows the severity classification according to ARIA for subgroups of respondents defined according to PG-MARJ (i.e., severest, severe, moderate, mild and asymptomatic). As a result, 79.7% of 468 respondents considered to have mild rhinitis and 67.2% of 67 respondents considered to have asymptomatic rhinitis according to PG-MARJ had moderate/severe

**Table 2** Distribution of disease type according to PG-MARJ

Total ($n$ = 3382)

| Severity | | Sneezing fit or rhinorrhea† ++++ | +++ | ++ | + | - |
|---|---|---|---|---|---|---|
| Nasal blockage | ++++ | 2.5% | 1.9% | 1.6% | 0.8% | 0.2% |
| | +++ | 6.7% | 5.6% | 5.3% | 2.1% | 0.3% |
| | ++ | 5.5% | 8.4% | 10.2% | 5.4% | 0.7% |
| | + | 3.0% | 5.2% | 10.4% | 7.5% | 1.0% |
| | - | 1.3% | 2.5% | 4.6% | 5.4% | 2.0% |

† Select more severe one, sneezing or rhinorrhea.
++++, Severest; +++, Severe; ++, Moderate; +, Mild; -, Asymptomatic.

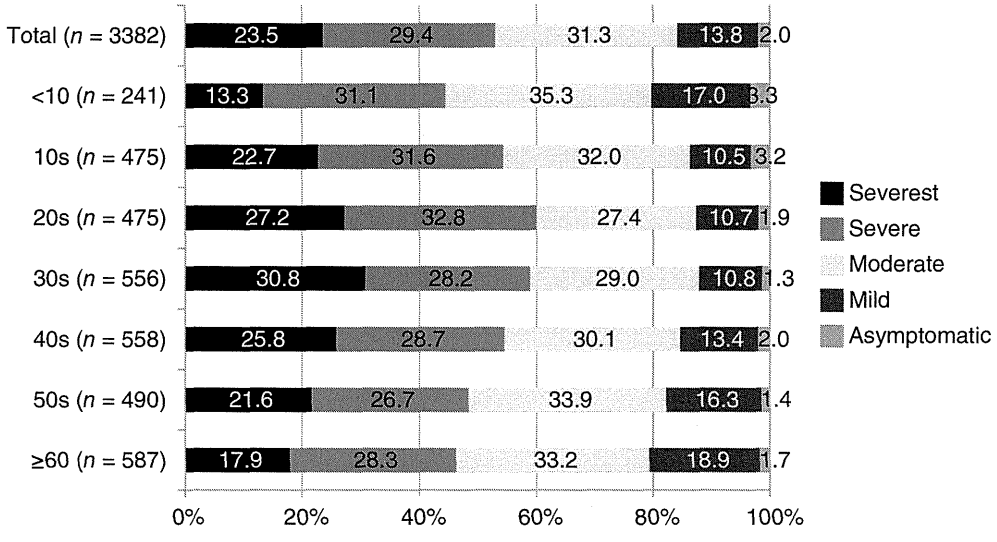


**Fig. 2** Severity by age group according to PG-MARJ.

rhinitis according to ARIA.

## AGREEMENT OF SEVERITY ASSESSMENT BETWEEN ARIA AND PG-MARJ

Respondents were grouped by severity according to PG-MARJ and ARIA into the following four categories: ARIA mild rhinitis and PG-MARJ severest/severe/moderate rhinitis, ARIA severe/moderate rhinitis and PG-MARJ mild/asymptomatic rhinitis, ARIA mild rhinitis and PG-MARJ mild/asymptomatic rhinitis, and ARIA severe/moderate rhinitis and PG-MARJ severest/severe/moderate rhinitis.

Thus, 117 respondents (3.5%) had mild/asymptomatic rhinitis according to both guidelines and 2661 respondents (78.7%) had moderate or more severe rhinitis. On the other hand, 418 respondents (12.4%) who had moderate/severe rhinitis according to ARIA had mild/asymptomatic rhinitis according to PG-MARJ, while 186 respondents (5.5%) who had mild rhinitis according to ARIA had moderate or more severe rhinitis according to PG-MARJ. This means that severity assessment by the two guidelines did not agree in 17.9% of the respondents. The distribution of the four categories by age group is shown in Figure 8. The disagreement rate was higher in the age group of <10 years (25.3%) and that of ≥60 years (22.0%).

## *DISCUSSION*

In order to determine the severity of symptoms in patients with springtime pollinosis, an Internet questionnaire survey was conducted between May 11 and May 18, 2011 when the cedar and cypress pollen dispersal season (from February to early May) had already ended, in individuals who had symptoms of pollinosis in the current pollinosis season and who had experienced those symptoms for at least two pollen seasons.

In Japan, the Practical Guideline for the Management of Allergic Rhinitis in Japan (PG-MARJ) was first established in 1993 to improve treatment for al-

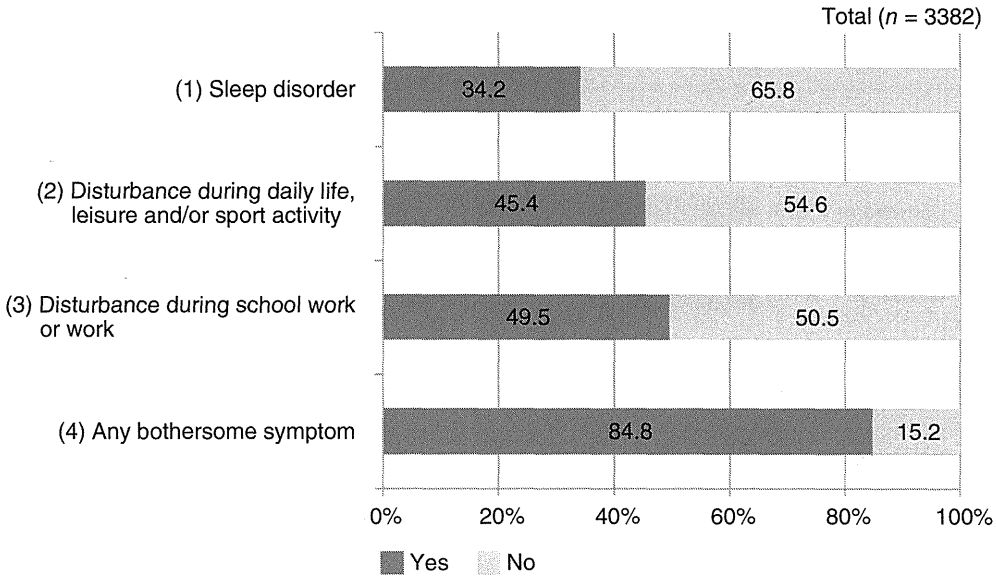


**Fig. 3** Disease's impact on daily life activities according to ARIA.

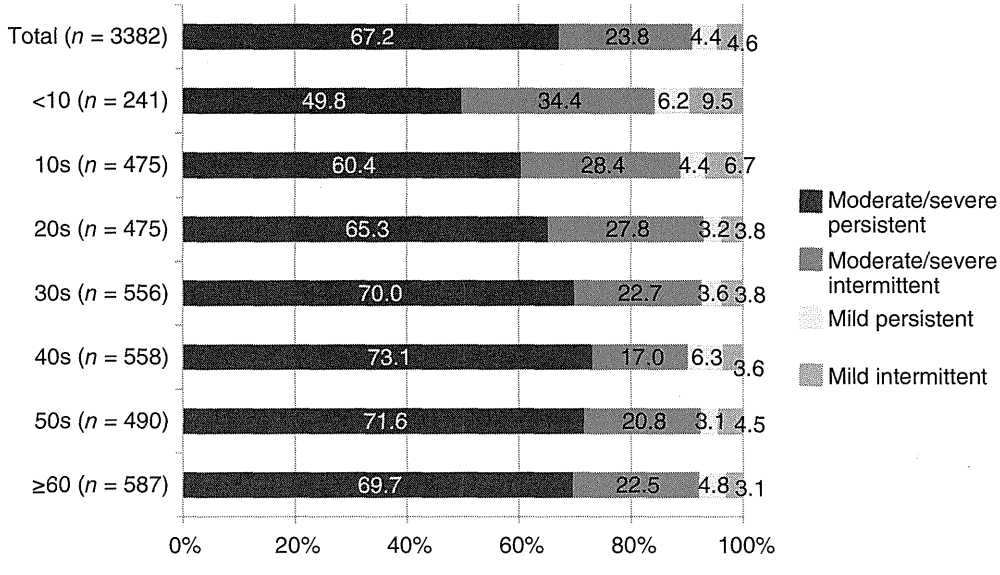


**Fig. 4** Severity by age group according to ARIA.

lergic rhinitis and now the 2009 revised version (Version 6) is being widely used.[2,4,5] The classification of severity in this guideline was used to assess the symptoms in the current season in each of the survey respondents. As a result, more than half of the respondents were considered to have severest or severe rhinitis and more than 80% to have moderate or more severe rhinitis. Severest or severe rhinitis was most prevalent in the respondents in their 20s and 30s (approximately 60%). In addition, more than 80% of all respondents had the three major symptoms (i.e., sneezing, rhinorrhea and nasal blockage). Approximately 2% of the respondents were considered to have asymptomatic rhinitis. Probably because the PG-MARJ classification of severity depends on the three major symptoms and does not take into account eye symptoms (itchy eyes and teary eyes), respondents who had eye symptoms alone may have been deemed to have asymptomatic rhinitis.

According to ARIA, due to symptoms of pollinosis, approximately one third of the respondents complained of sleep disorder, about half complained of disturbance during daily life, leisure and/or sport activities or school/work, and more than 80% complained of bothersome symptoms. According to ARIA, approximately 90% of the respondents had moderate/severe rhinitis and approximately 10% had mild rhinitis. Based on the duration of symptoms, ap-

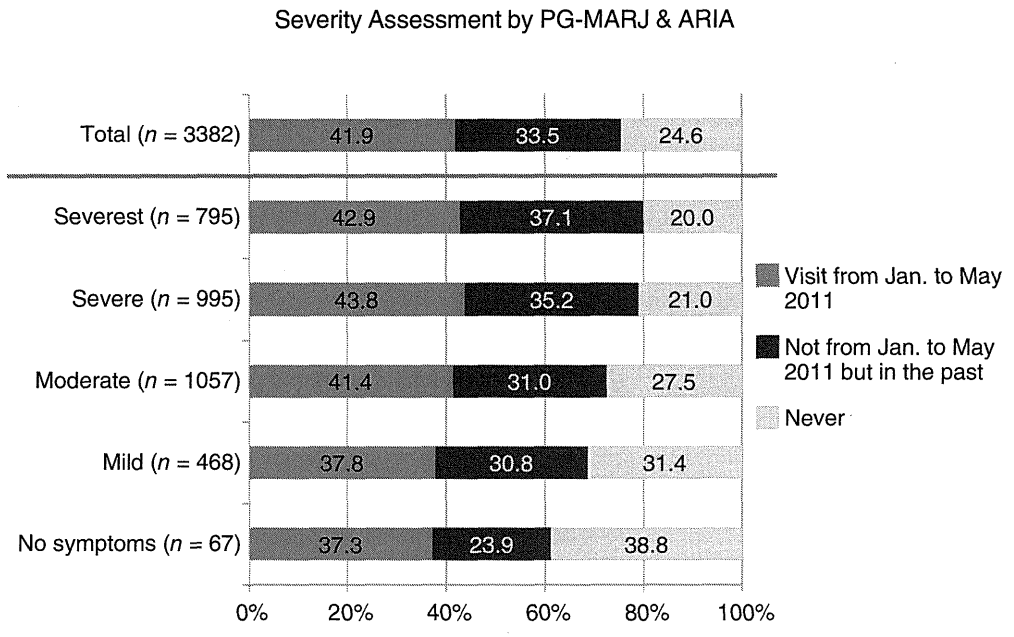


**Fig. 5** Proportion of respondents visiting a medical institution by severity according to PG-MARJ.

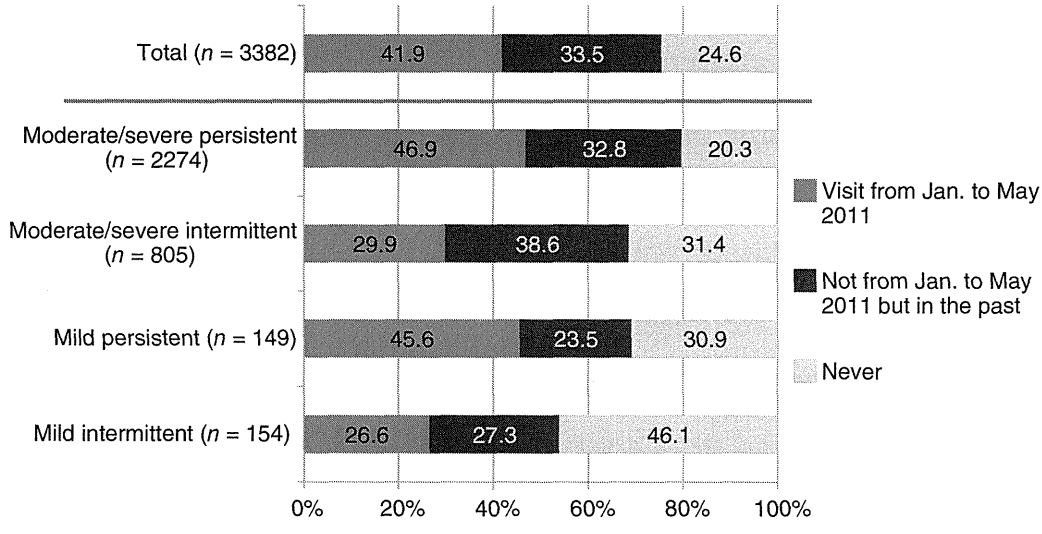


**Fig. 6** Proportion of respondents visiting a medical institution by severity according to ARIA.

proximately 70% of the respondents had persistent rhinitis and approximately 30% had intermittent rhinitis. Based on the combination of disease type and severity, 2274 respondents (67.2%) had moderate/severe persistent rhinitis, 805 (23.8%) moderate/severe intermittent rhinitis, 149 (4.4%) mild persistent rhinitis and 154 (4.6%) mild intermittent rhinitis. The analysis by age group indicated that younger respondents were less likely to have persistent rhinitis but more likely to have intermittent rhinitis.

It was naturally expected that the distribution of severity would vary among different causal antigens and among different geographical regions. A survey conducted in Europe where ARIA has been widely used reported that the proportion of mild intermittent, mild persistent, moderate/severe intermittent and moderate/severe persistent rhinitis was 10%, 14%, 17% and 59%, respectively.[6] This result showed less imbalance in different severity classes compared to the result of the present survey of Japanese cedar pollinosis. However, it has been reported that 90% of the patients seen at medical institutions had moderate/severe rhinitis[7,8] as is the case in the present survey. This means that the results may vary depending not only on antigens and geographical regions but also on the type of medical institutions where patients are seen. In the present survey, 41.9% of the 3382 respondents visited a medical institution between January and May 2011 and 17.3% used OTC drugs, while 42.5% did not visit any medical institution in the current pollen season and more than 40% of them used OTC drugs. Moreover, approximately 40% of the re-

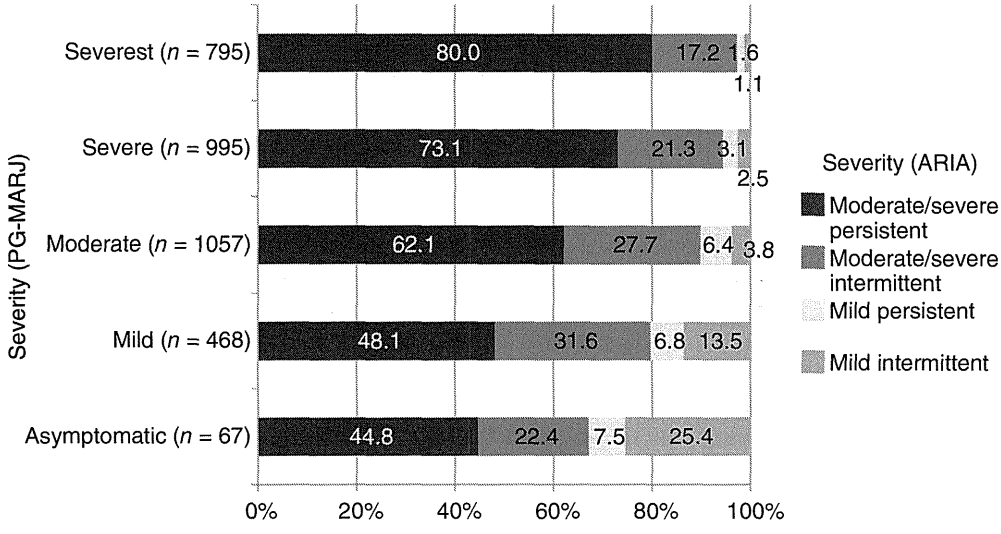


**Fig. 7** Correlation of severity assessment between PG-MARJ and ARIA.

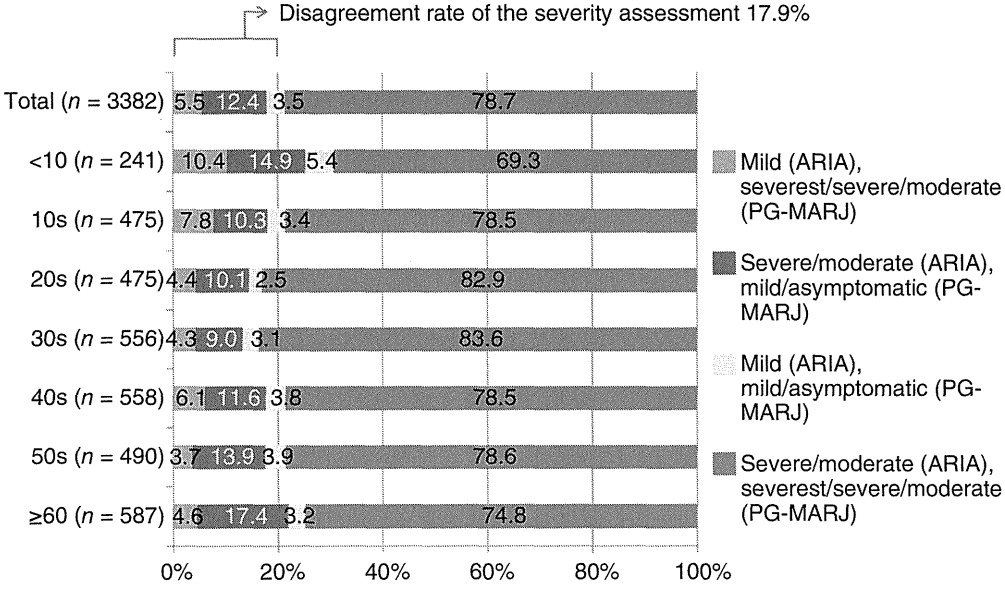


**Fig. 8** Agreement of severity assessment between ARIA and PG-MARJ.

spondents who used OTC drugs experienced drowsiness as a side effect (data not shown). The mainstream OTC oral drugs for the relief of pollinosis symptoms include first- and second-generation antihistaminics, which may cause drowsiness. The first-generation antihistaminics, which may cause severe drowsiness and dizziness and have a strong anticholinergic effect, are contraindicated in patients with glaucoma, benign prostatic hypertrophy and asthma. Concomitant use of other medications for the treatment of concurrent diseases or use of such OTC drugs without the supervision of a physician by patients who are engaged in driving or potentially hazardous activities is extremely dangerous and may cause decreased operating efficiency even during non-hazardous work or decreased academic performance. The results of the present survey indicate that it is necessary to improve awareness concerning allergic rhinitis (especially pollinosis) in the general public.

Severity assessment according to PG-MARJ and ARIA revealed higher prevalence of ARIA-defined persistent rhinitis and moderate/severe rhinitis in individuals with severer disease according to PG-MARG. This result indicates a positive correlation between the two guidelines. However, as high as approximately 80% and 70% of the respondents considered to have mild and asymptomatic rhinitis, respec-

tively, according to PG-MARG were deemed to have moderate/severe rhinitis according to ARIA. The disagreement rate of the severity assessment was approximately 18% in the overall population. The analysis by age group revealed higher disagreement rates of approximately 25% and 22% in the age group of <10 years and in that of ≥60 years, respectively. Probably because the severity classification in ARIA depends mainly on the impact on the quality of life (QOL) of patients, patients who had only mild symptoms but felt a large impact on daily life activities were likely to be classified as having moderate or more severe rhinitis. The classification in ARIA, which uses "presence or absence of bothersome symptoms" alone to assess the severity of nasal symptoms, may not accurately reflect the degree of nasal symptoms themselves.

The results of the present survey indicated that the majority of patients with Japanese cedar pollinosis had severe disease: more than 80% of the individuals who had symptoms of pollinosis during the current pollinosis season had moderate or more severe rhinitis according to PG-MARJ and more than 90% moderate/severe rhinitis according to ARIA. The classification system of ARIA may provide an incorrect diagnosis of severity since it may classify patients with severe nasal symptoms as having mild rhinitis. On the contrary, PG-MARG, which has been prepared based on the real conditions of allergic rhinitis in Japan, is considered to be a useful guideline in clinical practice.

## ACKNOWLEDGEMENTS

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# A critical role of IL-33 in experimental allergic rhinitis

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**Background: We reported previously that serum levels of IL-33 are significantly increased in patients with allergic rhinitis (AR). However, very little is known about the role of IL-33 for the development of AR.**

**Objective: We thought to develop a novel murine model of ragweed pollen–specific AR and examined the pathologic role for ragweed-induced IL-33 in the development of AR manifestation using IL-33–deficient *(il33$^{-/-}$)* mice.**

**Methods: Ragweed-immunized and ragweed-challenged mice were examined for early- and late-phase nasal responses. IL-33 protein expression in the nasal epithelial cells of the AR murine model and patients with AR were assessed by using confocal microscopy.**

**Results: After nasal challenge with ragweed pollen, ragweed-immunized wild-type mice manifested early-phase (sneezing) and late-phase (eosinophilic and basophilic accumulation) responses. In contrast, *il33*$^{-/-}$ and FcεRI$^{-/-}$ mice did not have both early- and late-phase AR responses. IL-33 protein was constitutively expressed in the nucleus of nasal epithelial cells and was promptly released into nasal fluids in response to nasal exposure to ragweed pollen. In human subjects we revealed constitutive expression of IL-33 protein in the nasal epithelial cells of healthy control subjects and downregulated expression of IL-33 protein in inflamed nasal epithelial cells of patients with AR. IL-33–stimulated mast cells and basophils contributed to the early- and late-phase AR manifestation through increasing histamine release and production of chemoattractants for eosinophils/basophils, respectively.**

**Conclusions: Ragweed pollen–driven endogenous IL-33 contributed to the development of AR responses. IL-33 might present an important therapeutic target for the prevention of AR. (J Allergy Clin Immunol 2012;130:184-94.)**

***Key words:*** *IL-33, allergic rhinitis, ragweed pollen, epithelial cells, sneezing, eosinophils, basophils, mast cells*

Allergic rhinitis (AR) is one of the most common allergic inflammatory diseases. Globally, more than 600 million persons have AR.[1] AR is divided into 2 categories: seasonal and perennial.[2] The prevalence of seasonal AR, pollinosis, is increasing in the developed world. Among allergenic weeds, ragweed (*Ambrosia* species) pollen is common and has been reported as the major source of airborne allergenic protein in the United States and many countries of central Europe.[3] At least 10% of the overall population in these countries is sensitized to ragweed, and the prevalence in atopic subjects is almost 50%.[3-5]

Nasal responses in patients with AR comprise 2 phases: IgE-dependent early-phase responses and $T_H2$ cytokine–dependent late-phase responses.[2,6,7] Clinical symptoms or signs, such as sneezing and rhinorrhea, occur as a result of the early-phase response within 5 to 30 minutes. Late-phase responses consist of congestion, fatigue, malaise, and irritability at 6 to 24 hours after exposure to an allergen. The major pathologic change associated with late-phase responses is influx of inflammatory cells, such as eosinophils, into the nasal mucosa.[2,6,7] The mechanisms underlying the development of bronchial asthma have been well analyzed by using a murine model. However, the precise mechanisms underlying the development of nasal responses in patients with AR have not been clearly defined.

IL-33, the latest member of the IL-1 family, is the ligand for ST2 (IL-33 receptor [IL-33R] α)[8] and shares the signaling pathway with IL-1 and IL-18.[8-10] However, unlike with IL-1 and IL-18, the protein maturation process is not necessary for IL-33 bioactivity. Full-length IL-33 has biological activity *in vivo*, and IL-33 is most likely released through cell necrosis or injury rather than cleavage by caspase.[11] Thus IL-33 has been referred to as an alarmin.[12] IL-33 was originally reported as a nuclear factor protein in endothelial cells of high endothelial venules[13]; hence it was initially called NF-HEV. Indeed, IL-33 is constitutively expressed and localized in the nucleus of epithelial and endothelial cells from various tissues.[14,15]

IL-33 has the capacity to induce $T_H2$ cytokine production in $T_H2$ cells,[8,16] mast cells,[17] basophils,[16,18] eosinophils,[19,20] and newly identified innate immune cells (natural helper cells and nuocytes),[21,22] suggesting that IL-33 has the potential to induce $T_H2$ cytokine–mediated allergic inflammation.[23] Indeed, IL-33

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*Abbreviations used*
AR: Allergic rhinitis
DNP: 2,4-Dinitrophenyl
CTMC: Connective tissue–type mast cell
FITC: Fluorescein isothiocyanate
*il33*$^{-/-}$: IL-33 deficient
IL-33R: IL-33 receptor
JC: Japanese cedar
MCP-1: Monocyte chemotactic protein 1
MIP-1α: Macrophage inflammatory protein 1α
MMC: Mucosal mast cell
mMCP-8: Murine mast cell protease 8
OVA: Ovalbumin
PE: Phycoerythrin
SSC: Side scatter
WT: Wild-type

is implicated in asthma,[16,18] allergic conjunctivitis,[19] and anaphylactic responses.[24] Furthermore, we showed previously that the serum IL-33 level is significantly increased in Japanese patients with seasonal AR and revealed a significant association between susceptibility to AR and IL-33 polymorphism.[25]

Given the difficulty in examining the mechanisms in human subjects, a murine model of AR is essential. However, there is no appropriate murine model of AR, especially for seasonal AR. Here we established a novel murine model of ragweed-specific AR and examined the pathologic role for endogenous IL-33 in the induction of early- and late-phase AR manifestation by using IL-33–deficient (*il33*$^{-/-}$) mice.

## METHODS

For more information, see the Methods section in this article's Online Repository at www.jacionline.org.

### Mice

The generation of *il33*$^{-/-}$ mice is detailed in our separate report.[26] *il33*$^{-/-}$ mice (129SvJ × C57BL/6) were backcrossed for 7 generations onto BALB/c mice, and their littermate controls (*il33*$^{+/+}$) were used for the experiments.

### Human samples

A total 10 patients with AR and 5 healthy subjects were recruited from the University Hospital, Kyoto Prefectural University of Medicine; 13 patients with AR and 11 healthy subjects were recruited from the University of Fukui Hospital. Demographic and clinical characteristics of the control subjects and patients are summarized in Tables E1 and E2 in this article's Online Repository at www.jacionline.org. For more information, see the Methods section in this article's Online Repository.

### Experimental AR by active immunization

Mice were immunized with a mixture of ragweed pollen (100 μg in 200 μL) and aluminum hydroxide hydrate gel (1 mg in 200 μL; Sigma-Aldrich, St Louis, Mo) by means of intraperitoneal injection on day 0 and with ragweed/PBS (100 μg in 200 μL) by means of intraperitoneal injection on day 7. A week after the boost, mice (5 mice per group) were challenged by means of nasal administration of ragweed pollen (1 mg in 20 μL of PBS) or PBS (20 μL) for 4 consecutive days. Immediately after each nasal challenge, the frequency of sneezing was counted in a blinded manner for 10 minutes. Peripheral blood was collected from the inferior vena cava 24 hours after the final nasal challenge, and then sera were prepared by using centrifugation. The mice were killed, and the nose and cervical lymph nodes were isolated for further histologic and immunologic analysis.

### Flow cytometry and cell purification

Bone marrow–derived connective tissue–type mast cells (CTMCs), mucosal mast cells (MMCs), and basophils were prepared as described previously.[16,27,28] The purity of each population was greater than 97%.

### Statistics

Statistical significance was calculated with the 2-tailed Student *t* test. *P* values of less than .05 were considered statistically significant.

## RESULTS

### Establishment of ragweed-immunized ragweed-induced AR

We first generated a murine model of ragweed-specific AR. We immunized BALB/c background *il33*$^{+/+}$ mice with ragweed pollen by means of sequential intraperitoneal injection of ragweed/alum and ragweed/PBS. Then we challenged the mice by means of nasal administration of ragweed pollen or PBS for 4 consecutive days. We counted the frequency of sneezing over a 10-minute period immediately after the last nasal challenge. Compared with PBS-challenged control mice, ragweed-challenged mice showed a significant increase in the frequency of sneezing (Fig 1, *A*), which suggests that the ragweed pollen challenge induces immediate-type AR, possibly in an IgE-dependent manner. Indeed, compared with PBS-challenged mice, ragweed-challenged mice showed significantly increased total and ragweed-specific IgE levels in their sera when measured 1 day after the final challenge ($P < .005$; Fig 1, *B*).

Histologic analysis showed a multilayered epithelium, goblet cell hyperplasia, and prominent accumulation of eosinophils in the nasal lateral mucosa, nasal turbinate, and nasal septal mucosa of ragweed-challenged mice but not of PBS-challenged mice (Fig 1, *C-E*, and see Figs E1-E3 in this article's Online Repository at www.jacionline.org). In addition, increased numbers of eosinophils in the cervical lymph nodes were observed (see Fig E4 in this article's Online Repository at www.jacionline.org). Most nasal eosinophils (Siglec-F$^+$ cells) expressed ST2 (IL-33Rα; Fig E1, *C*). In all, this ragweed-specific AR murine model mimics the major features of human AR, especially ragweed-induced pollinosis, in terms of nasal symptoms and histologic changes after intranasal exposure to ragweed pollen.

### *il33*$^{-/-}$ mice did not mount $T_H2$/IgE responses on ragweed challenge

To clarify the physiologic role of endogenous IL-33 in AR, we generated BALB/c background *il33*$^{-/-}$ mice.[26] Compared with *il33*$^{+/+}$ mice, ragweed-immunized *il33*$^{-/-}$ mice showed a significant reduction in the frequency of sneezing, total and ragweed-specific IgE response, and accumulation of eosinophils in the nasal mucosa and cervical lymph nodes after nasal administration of ragweed pollen. In addition, histologic analysis revealed that *il33*$^{-/-}$ mice showed a diminished degree of multilayer formation in the epithelium and goblet cell hyperplasia in the nasal mucosa (Fig 1, *A-D*, and see Figs E2-E4). Like *il33*$^{+/+}$ mice, however, ragweed-immunized and PBS-challenged *il33*$^{-/-}$ mice evidenced considerably increased total IgE levels in their sera

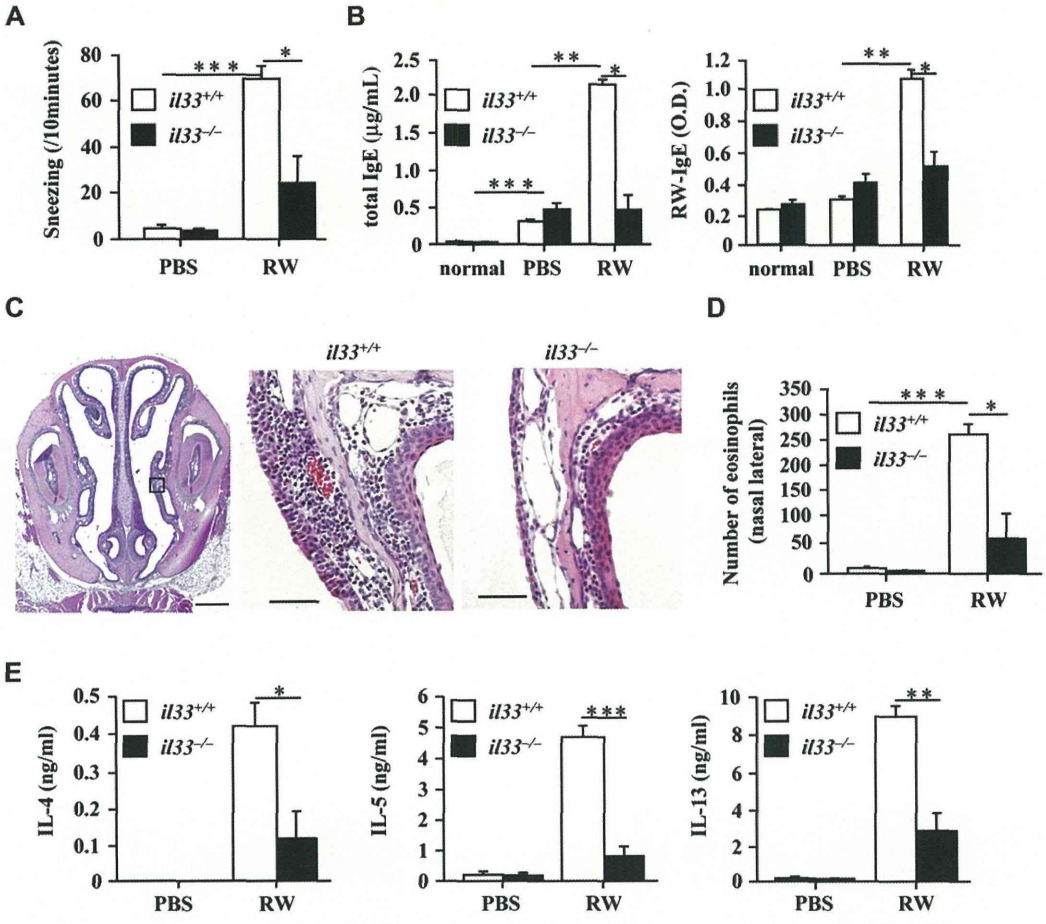


**FIG 1.** *il33*$^{-/-}$ mice do not induce ragweed *(RW)*–induced AR. Ragweed-immunized mice were nasally challenged with PBS or ragweed. **A,** Number of sneezes. **B,** Total and ragweed-specific IgE levels in serum. **C,** Hematoxylin and eosin staining of the nose from ragweed-immunized ragweed-challenged mice. **D,** Number of eosinophils in nasal mucosa. **E,** Cytokine production by cervical lymph node cells. Data are representative of 3 independent experiments (means and SEMs of 5 mice). *$P < .05$, **$P < .005$, and ***$P < .001$. *Bar* = 500 μm (Fig 1, *C, left*) and 50 μm (Fig 1, *C, middle* and *right*).

compared with those seen in nonimmunized *il33*$^{-/-}$ mice (Fig 1, *B*), suggesting that *il33*$^{-/-}$ mice have the capacity to develop $T_H2$/IgE responses on immunization but have a markedly diminished capacity to mount $T_H2$/IgE responses on ragweed challenge. Indeed, cervical lymph node cells from ragweed-challenged *il33*$^{-/-}$ mice showed a markedly diminished production of IL-4, IL-5, and IL-13 on stimulation with ragweed extract protein *in vitro* compared with those from *il33*$^{+/+}$ mice (Fig 1, *E*). These results clearly indicate that endogenous IL-33 contributes to induction of both early- and late-phase AR manifestation.

## Exposure to ragweed pollen induced IL-33 release from the nasal epithelium

We next examined the expression of IL-33 in nasal epithelial cells and the secretion of IL-33 in response to nasal exposure to ragweed pollen. Immunohistochemical analysis revealed that IL-33 is constitutively expressed in the nucleus of nasal epithelial cells (Fig 2, *A* and *B*). IL-33 was not detected in the nasal mucosa of *il33*$^{-/-}$ mice, which indicates the specificity of this staining for IL-33 protein (Fig 2, *A*). The expression of IL-33 in the nucleus of nasal epithelial cells from mice that had been continuously challenged with ragweed pollen for 4 days was considerably lower than that from mice challenged with PBS (Fig 2, *C*), suggesting that exposure to ragweed pollen reduced IL-33 expression by causing epithelial cells to secrete IL-33.

To examine this possibility, we performed a time-course analysis of IL-33 expression in nasal epithelial cells after ragweed challenge. We found that nasal IL-33 expression was promptly downregulated and became very faint 1 hour after ragweed administration, but it recovered gradually thereafter (Fig 2, *D* and *E*). Consistent with this observation, IL-33 protein levels increased promptly in nasal lavage fluid of naive wild-type (WT) mice but not *il33*$^{-/-}$ mice after ragweed administration (Fig 2, *F*, and data not shown). Thus nasal exposure to ragweed pollen promptly induces endogenous IL-33 production from nasal epithelial cells, and resultant IL-33 subsequently contributes to induction and augmentation of AR manifestation.

## IL-33 stimulated FcεRI$^+$ mast cells to increase histamine release

In patients with AR, histamine released from activated mast cells and basophils has an important role in the induction of sneezing.[29,30] Thus we next examined the role of mast cells and

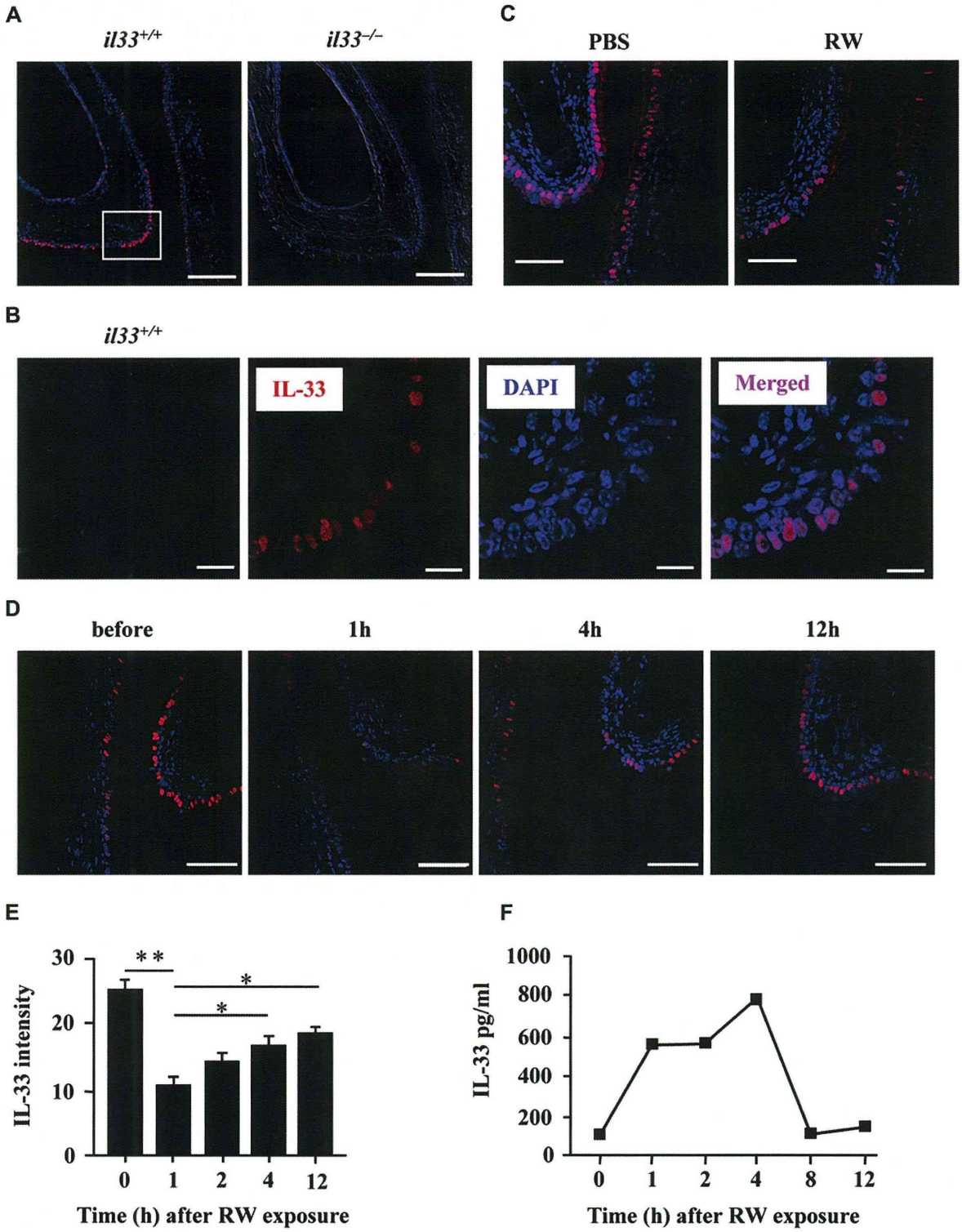


**FIG 2.** Nasal administration of ragweed *(RW)* pollen induces IL-33 release from nasal epithelial cells. **A-C,** Immunofluorescence staining of nose stained for IL-33 *(red)* and 4′-6-diamidino-2-phenylindole dihydrochloride *(blue)*. Fig 2, *A* and *B*, Naive nice. Fig 2, *C*, Ragweed-immunized and PBS- or ragweed-challenged mice. **D-F,** Naive WT mice were nasally administered single ragweed challenge and killed at the indicated time. Fig 2, *D*, Staining of IL-33. Fig 2, *E*, Quantitative image analysis of stained IL-33. Fig 2, *F*, IL-33 protein level in nasal lavage fluid. Data are representative of 3 independent experiments (3 mice per time point). $*P < .005$ and $**P < .0001$. *Bar* = 50 μm.

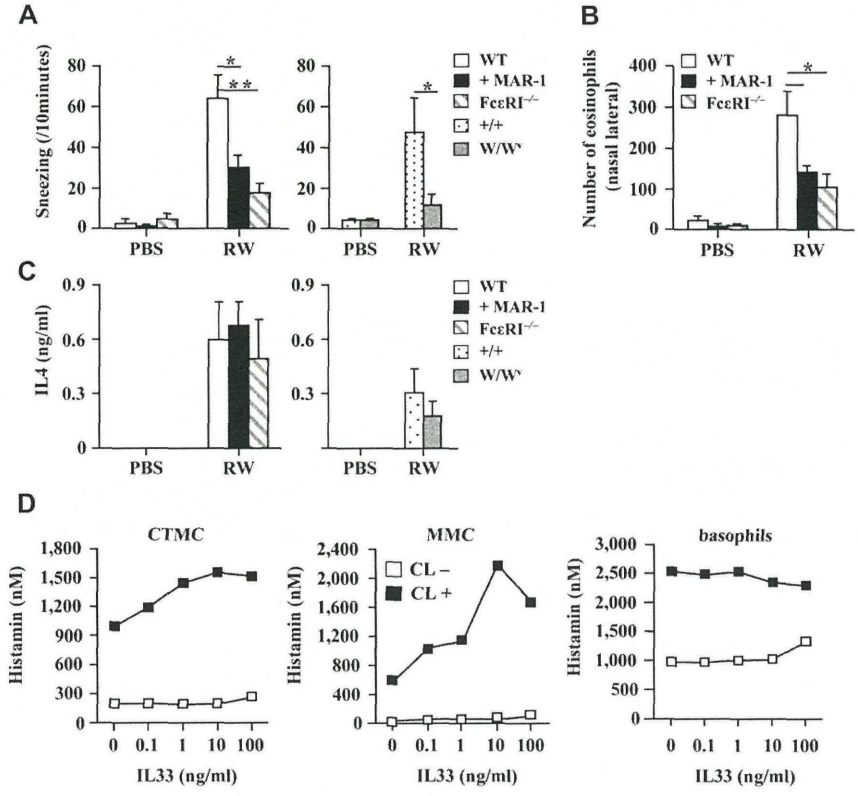


**FIG 3.** IL-33 induces histamine release from FcεR1$^+$ cells. **A-C,** Ragweed *(RW)*–immunized WT, basophil-depleted *(+MAR-1)*, FcεRI$^{-/-}$, WBB6F1-$^{+/+}$ *(+/+)*, and WBB6F1-W/W$^v$ *(W/W$^v$)* mice were challenged with PBS or ragweed. Fig 3, *A*, Number of sneezes. Fig 3, *B*, Number of eosinophils in nasal mucosa. Fig 3, *C*, IL-4 production by cervical lymph node cells. Data are representative of 2 independent experiments (means and SEMs of 5 mice). $*P < .05$ and $**P < .01$. **D,** ELISA of histamine. *CL+*, With FcεRI cross-linkage; *CL−*, without FcεRI cross-linkage. Data are representative of 5 independent experiments.

basophils in experimental AR. For this purpose, we used mast cell–deficient WBB6F1-W/W$^v$ mice, basophil-depleted mice,[28] and FcεRI$^{-/-}$ mice. Compared with ragweed-immunized control mice, ragweed immunization of all of these mice evidenced a significant diminishing of sneezing frequency after nasal exposure to ragweed pollen (Fig 3, *A*). In addition to sneezing, eosinophilic accumulation in the nasal mucosa was also significantly reduced in basophil-depleted and FcεRI$^{-/-}$ mice, suggesting the importance of basophils or basophils plus mast cells for eosinophilic accumulation (Fig 3, *B*). Nevertheless, cervical lymph node cells from ragweed-challenged basophil-depleted mice, FcεRI$^{-/-}$ mice, or WBB6F1-W/W$^v$ mice produced amounts of $T_H2$ cytokines comparable with those seen in control mice on stimulation *in vitro* (Fig 3, *C*). These results suggest that in addition to $T_H2$ cells, activated FcεRI$^+$ mast cells and basophils might contribute to both early-phase (sneezing) and late-phase (eosinophilic accumulation) responses in AR.

To reveal the mechanism whereby *il33*$^{-/-}$ mice suppressed the induction of sneezing (Fig 1, *A*), we examined the role of IL-33 in histamine release from mast cells and basophils. Mast cells are generally classified into 2 populations, CTMCs and MMCs, both of which exist in the nasal membranes of patients with AR.[31-36] It is controversial whether mast cells (and if so which type) or basophils play the crucial role in AR.[31,33,35,37,38] Thus we developed and purified CTMCs, MMCs, and basophils from murine bone marrow cells (see Fig E5 in this article's Online Repository at www.jacionline.org) and examined their capacity to release histamine in response to IL-33 *in vitro*. Stimulation with IL-3 induced production of histamine from CTMCs and basophils but not from MMCs; additional IL-33 stimulation did not enhance histamine release from these cells (Fig 3, *D*). In contrast, cross-linkage of FcεRI significantly enhanced histamine release from CTMCs, MMCs, and basophils. Furthermore, IL-33 dose-dependently augmented histamine release from CTMCs and MMCs but not from basophils, although all these cells express IL-33Rα (Fig 3, *D*, and see Fig E5). These results suggest that ragweed pollen–induced endogenous IL-33 stimulates CTMCs and MMCs to increase histamine release under the condition of cross-linkage of FcεRI with IgE–ragweed pollen.

## *il33*$^{-/-}$ mice showed diminished ragweed-induced nasal accumulation of basophils

Histologic examination revealed that there were very few basophils in the nasal mucosa of ragweed-immunized PBS-challenged mice. However, ragweed challenge significantly increased the number of basophils in the nasal mucosa at 24 hours after the final challenge, particularly in the posterior section of the nose ($18.5 \pm 4.5$ in the anterior section compared with $34.5 \pm 10.6$ in the posterior section) of ragweed-immunized mice, as illustrated by immunohistochemical staining with an mAb specific for murine basophils (Fig 4, *A* and *B*).[39] We found that the degree of basophil accumulation in the nasal mucosa of *il33*$^{-/-}$ or FcεRI$^{-/-}$ mice was significantly lower than in control mice (Fig 4, *B*).

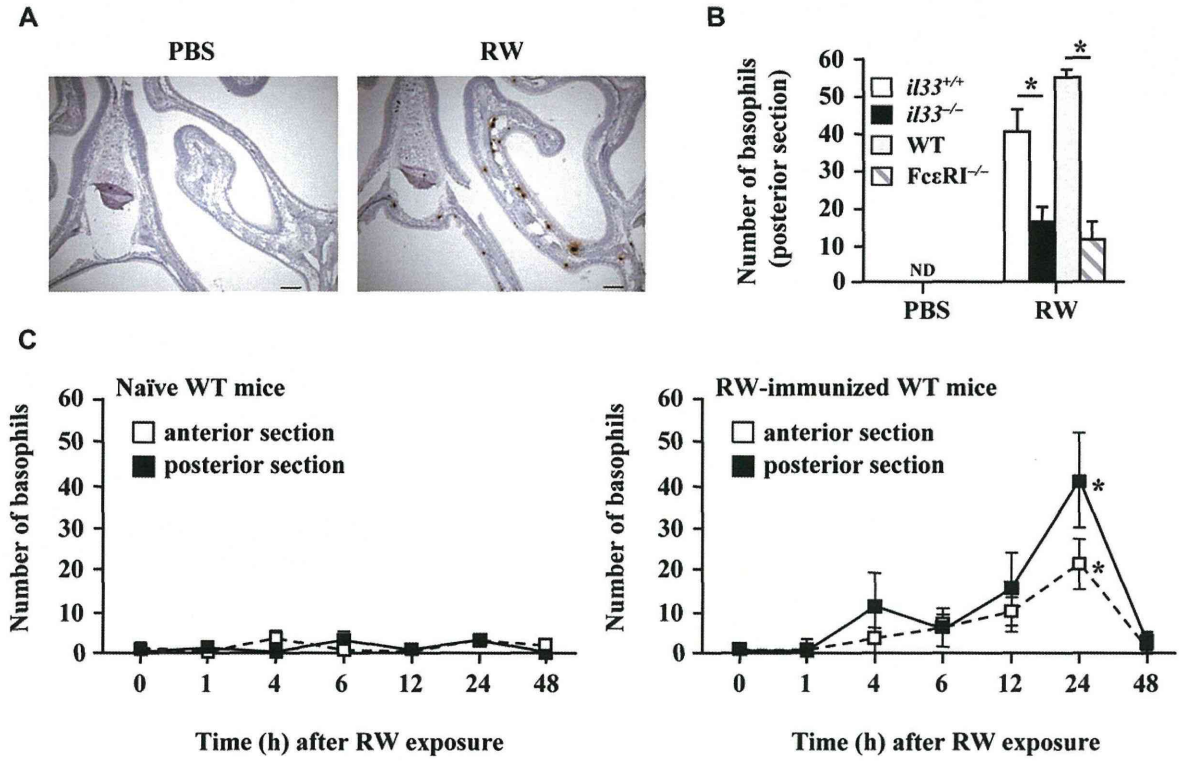


**FIG 4.** Ragweed *(RW)*–induced endogenous IL-33 regulates nasal accumulation of basophils. **A** and **B**, Ragweed-immunized mice were nasally challenged with PBS or ragweed. Fig 4, *A*, Immunohistochemical staining for basophils in the nose from *il33*$^{+/+}$ mice. *Bar* = 100 μm. Fig 4, *B*, Number of basophils in the nose. Fig 4, *C*, Naive or ragweed-immunized WT mice were nasally administered single ragweed. Kinetics of the number of basophils in the nose (3 mice per time point) are shown. Data are representative of 2 or 3 independent experiments. Means and SEMs of 3 mice are shown. Fig 4, *B*, **P* < .05. Fig 4, *C*, **P* < .05 compared with before ragweed exposure (0 hours).

We studied the kinetics of accumulation of basophils in the nasal mucosa after challenge with ragweed pollen. Ragweed-immunized WT mice had significantly increased basophil accumulation in the nasal mucosa, especially in the posterior section of the nose, at 4 hours, and this peaked at 24 hours after provocation; naive mice did not show this (Fig 4, *C*). Similar to basophils, the number of eosinophils in the nasal mucosa increased, whereas the number of CTMCs or MMCs decreased after nasal administration of ragweed pollen into ragweed-immunized mice (see Fig E6 in this article's Online Repository at www.jacionline.org). However, we were able to detect degranulated CTMCs in the nasal mucosa at 4 hours after ragweed challenge (data not shown), which suggests that both basophils and mast cells might be the early IL-33–responding cells. Taken together, these results suggest that basophil accumulation in AR is regulated by ragweed-induced endogenous IL-33, the IgE/FcεRI pathway, or both.

## *il33*$^{-/-}$ mice developed AR on ragweed plus IL-33 challenge

Ragweed-immunized *il33*$^{-/-}$ mice were nasally challenged with ragweed and IL-33 to examine the role of FcεRI$^{+}$ cells stimulated by IL-33 plus FcεRI cross-linkage *in vivo*. As seen in Fig 1, *B*, ragweed-immunized and ragweed-challenged *il33*$^{-/-}$ mice evidenced a considerably increased total IgE level but did not have AR responses (Fig 5). In contrast, ragweed-immunized and ragweed plus IL-33–challenged *il33*$^{-/-}$ mice manifested early- and late-phase nasal responses and mounted $T_H2$ responses (Fig 5). These results further substantiate that IL-33 plus FcεRI cross-linkage is essential to the development of AR.

## IL-33 stimulated FcεRI$^{+}$ cells to produce chemoattractants for both eosinophils and basophils

Accumulation of both eosinophils and basophils in the nasal mucosa in ragweed-immunized and ragweed-challenged *il33*$^{-/-}$ or FcεRI$^{-/-}$ mice was significantly lower than that seen in control mice (Fig 1, *D*, Fig 3, *B*, and Fig 4, *B*), which suggests that both IL-33 and FcεRI$^{+}$ cells are essential for the recruitment of eosinophils, basophils, or both. The role of cytokines and chemokines in the accumulation of eosinophils and basophils in inflamed tissue has been well studied.[40,41] Thus we examined the capacity of CTMCs, MMCs, and basophils to produce cytokines and chemokines in response to IL-33 plus cross-linkage of FcεRI. Cross-linkage of FcεRI on CTMCs and MMCs in the presence of IL-3 markedly induced production of IL-1β and eotaxin (Fig 6). However, CTMCs and MMCs increased production of IL-9, IL-13, GM-CSF, RANTES, macrophage inflammatory protein 1α (MIP-1α), and monocyte chemotactic protein 1 (MCP-1) when additionally stimulated with IL-33 (Fig 6). As reported in our previous article,[16,28] basophils strongly produced all the cytokines and chemokines that we measured. Because one set of chemokines (eotaxin, RANTES, and MIP-1α) and the other set (RANTES, MIP-1α, and MCP-1) have been shown to act,

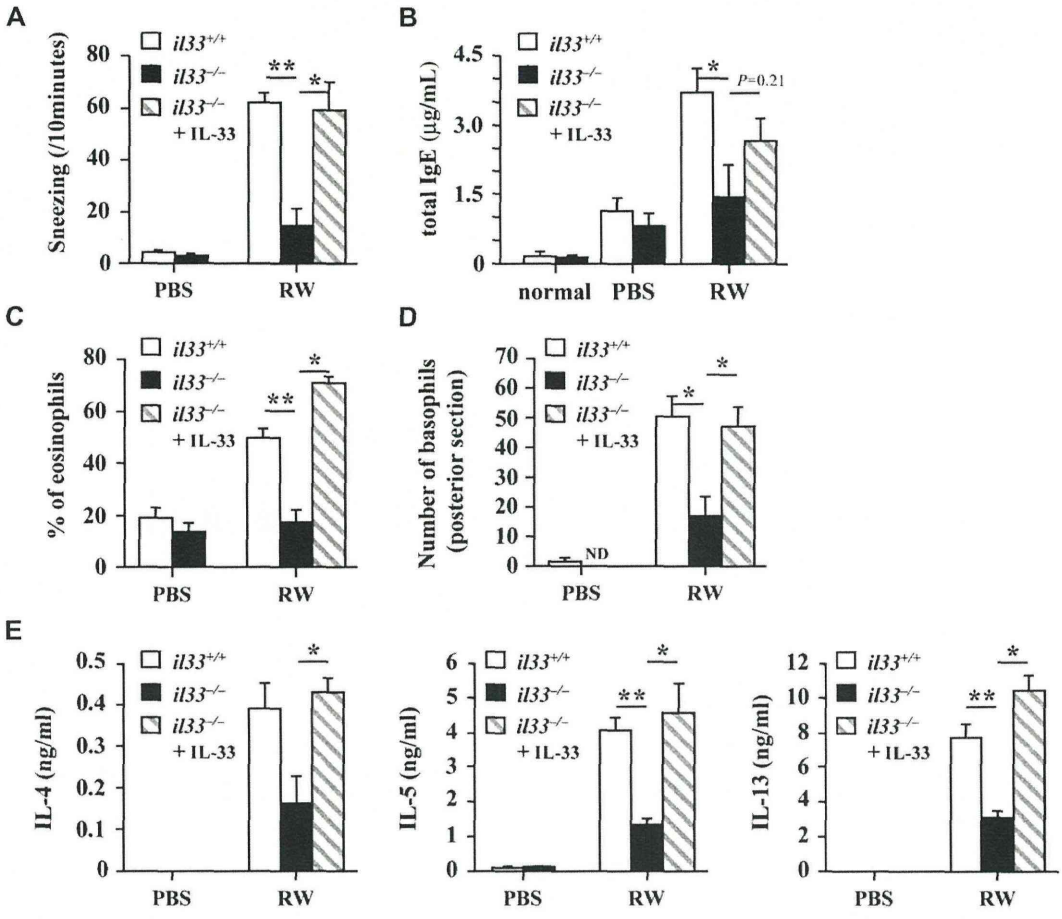


**FIG 5.** Ragweed *(RW)* plus IL-33–challenged *il33*$^{-/-}$ mice developed AR. Ragweed-immunized mice were nasally challenged with PBS or ragweed ± IL-33 (1 μg). **A,** Number of sneezes. **B,** Total IgE levels in serum. **C,** Percentage of eosinophils in cervical lymph nodes. **D,** Number of basophils in nasal mucosa. **E,** Cytokine production by cervical lymph node cells. Data are means and SEMs of 5 mice. *$P < .05$ and **$P < .005$.

respectively, as eosinophil[41,42] and basophil[40,43,44] chemotactic factors, ragweed pollen–driven endogenous IL-33 seems to play an important role in the recruitment of eosinophils and basophils by inducing the production of chemoattractants for both these types of cells. In addition, ragweed-immunized WT mice induced the expression of genes encoding MIP-1α and MCP-1 in the nasal mucosa, which peaked at 4 hours and decreased gradually after the ragweed challenge, whereas ragweed-immunized *il33*$^{-/-}$ mice did not do so (data not shown). These results further substantiate that ragweed pollen–driven endogenous IL-33 contributes to the temporal recruitment of inflammatory cells into the nasal mucosa through the induction of chemoattractants.

### IL-33 expression was diminished in nasal epithelial cells of patients with AR

Finally, IL-33 expression was analyzed in nasal epithelial cells taken from patients with AR and healthy control subjects to determine the relevance of the findings in the AR murine model to patients with AR (see Tables E1 and E2). Although IL-33 protein was strongly expressed in the nucleus of nasal epithelial cells from healthy subjects, diminished or even undetectable expression of IL-33 was found in nasal epithelial cells in patients with AR (Fig 7, *A*). A quantitative analysis showed significantly reduced IL-33 expression in nasal epithelial cells in patients with AR (Fig 7, *B*). On the other hand, compared with healthy control subjects, IL-33 mRNA expression in nasal epithelial cells from patients with AR significantly increased during the pollen season (Fig 7, *C*). These results indicate that as in the AR murine model, IL-33 protein expression was significantly reduced in inflamed nasal epithelial cells in patients with AR. However, IL-33 mRNA expression in nasal epithelial cells from patients with AR was considerably upregulated. Taken together, these results indicate the involvement of nasal IL-33 in the induction of AR.

## DISCUSSION

We first demonstrated that compared with control mice, ragweed-immunized and ragweed-challenged *il33*$^{-/-}$ mice showed a significant reduction in the frequency of sneezing, total and ragweed-specific IgE response, and accumulation of eosinophils and basophils in the nasal mucosa. These mice evidenced a diminished capacity of their cervical lymph node T cells to produce $T_H2$ cytokines *in vitro*. Furthermore, histologic examination revealed only modest changes in the noses of ragweed-immunized, ragweed-challenged *il33*$^{-/-}$ mice. Thus IL-33 is an essential molecule in the development of ragweed-induced AR. In addition to these results, endogenous IL-33 is critically involved in the development of ovalbumin (OVA)–specific

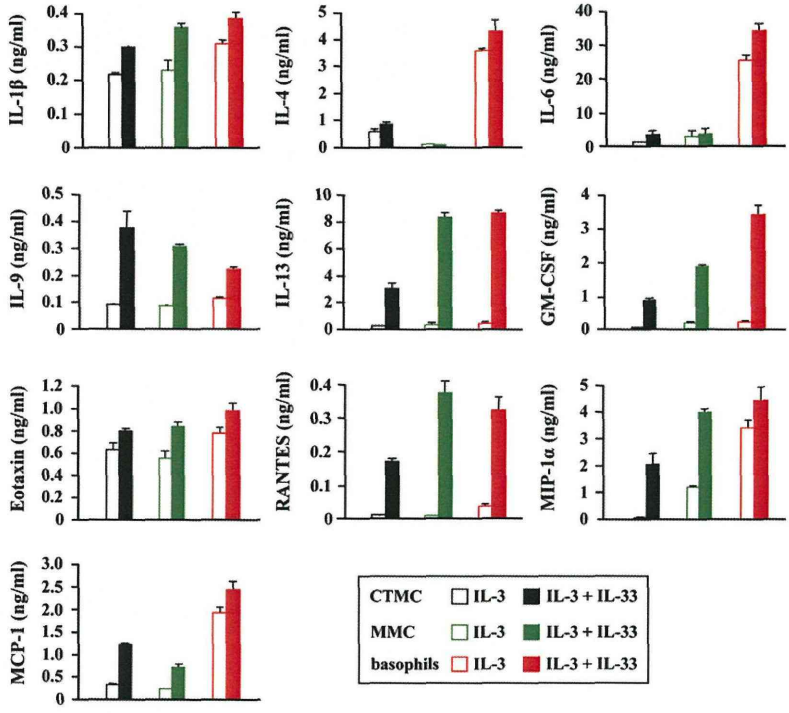


**FIG 6.** IL-33 induces the production of chemoattractants for eosinophils and basophils from FcεRI$^+$ cells. Cytokines and chemokines produced by CTMCs, MMCs, or basophils stimulated for 16 hours with IL-3 alone or IL-3 plus IL-33 with FcεRI cross-linkage. Data are representative of 3 independent experiments (means and SEMs).

$T_H2$-type immune responses,[45,46] which suggests that IL-33 is not specific to the ragweed-induced response.

Recruitment of $T_H2$ cells to the site of allergen challenge is a key step in the induction of AR. We found that AR mice showed increased capacity of their cervical lymph node T cells to produce $T_H2$ cytokines. Furthermore, we could detect $CD4^+ST2^+$ T cells ($T_H2$ cells)[47] in the nasal mucosa in ragweed-immunized, ragweed-challenged *il33*$^{+/+}$ mice but not in *il33*$^{-/-}$ mice (data not shown). The mechanism underlying reduced $T_H2$/IgE responses on ragweed pollen challenge in *il33*$^{-/-}$ mice is not clear. We propose the possibility that ragweed-induced endogenous IL-33 might enhance interaction between antigen-presenting cells and $T_H2$ cells in the cervical lymph nodes. Alternatively, IL-33 might enhance recruitment of $T_H2$ cells into the cervical lymph nodes. Indeed, it has been reported that IL-33 is a selective $T_H2$ cell chemoattractant.[48]

Next, we demonstrated that IL-33 protein is constitutively expressed in the nucleus of nasal epithelial cells and that these IL-33 expressions diminished within 1 hour after exposure to ragweed pollen. By contrast, IL-33 protein levels promptly increased in nasal lavage fluid. This is the first report to demonstrate that pollen grains induce IL-33 protein *in vivo*. At the same time, we were able to reveal constitutive IL-33 expression in the nasal epithelial cells of healthy control subjects and downregulated expression of IL-33 in inflamed nasal epithelial cells of patients with AR. However, IL-33 mRNA expression in nasal epithelial cells from patients with AR was not reduced but significantly upregulated during the pollen season, suggesting that enhanced extracellular IL-33 release was associated with reduced IL-33 protein expression in inflamed nasal epithelial cells, as in the AR murine model. We previously reported that the serum level of IL-33 is significantly increased in patients with AR,[25] which suggests the importance of measuring IL-33 levels in nasal lavage fluid after provocation of allergens in patients with AR.

Unlike IL-1β and IL-18, full-length IL-33 has biological activity and loses its activity after cleavage with caspases.[11] It is believed that epithelial cells produce IL-33 when they become necrotic or injured.[12] At present, we have no data about necrosis of epithelial cells by ragweed pollen. Recently, Kouzaki et al[49] demonstrated that in response to a fungal antigen, *Alternaria alternata*, bronchial epithelial cells translocate nuclear IL-33 and actively release full–length proform IL-33. Ragweed pollen is known to contain antigenic and enzymatic proteins.[50] Further study is needed to define the mechanisms for IL-33 release by ragweed pollen.

We demonstrated the importance of basophils and mast cells in the induction of AR. Histamine released from activated mast cells and basophils has been recognized as one of the most important chemical mediators for sneezing in patients with AR.[29,30] We demonstrated that IL-33, together with cross-linkage of FcεRI, dose-dependently increased the production of histamine by CTMCs and MMCs but not by basophils. However, human basophils increase further histamine release on additional IL-33 stimulation.[51] Thus endogenous IL-33 enhances AR by stimulation of FcεRI$^+$ cells, and it becomes a therapeutic target molecule. Indeed, we found that treatment with recombinant human ST2-Fc chimera protein (the decoy receptor of IL-33)[9,52] into ragweed-immunized mice during ragweed challenge significantly reduced the frequency of sneezing (data not shown).

Several reports have shown that the numbers of CTMCs, MMCs, and basophils are increased, decreased, or unchanged in the nasal mucosa of patients with AR examined after nasal allergen provocation or during the pollen season.[31,33,35,37,38] In our AR murine model the numbers of CTMCs or MMCs in the

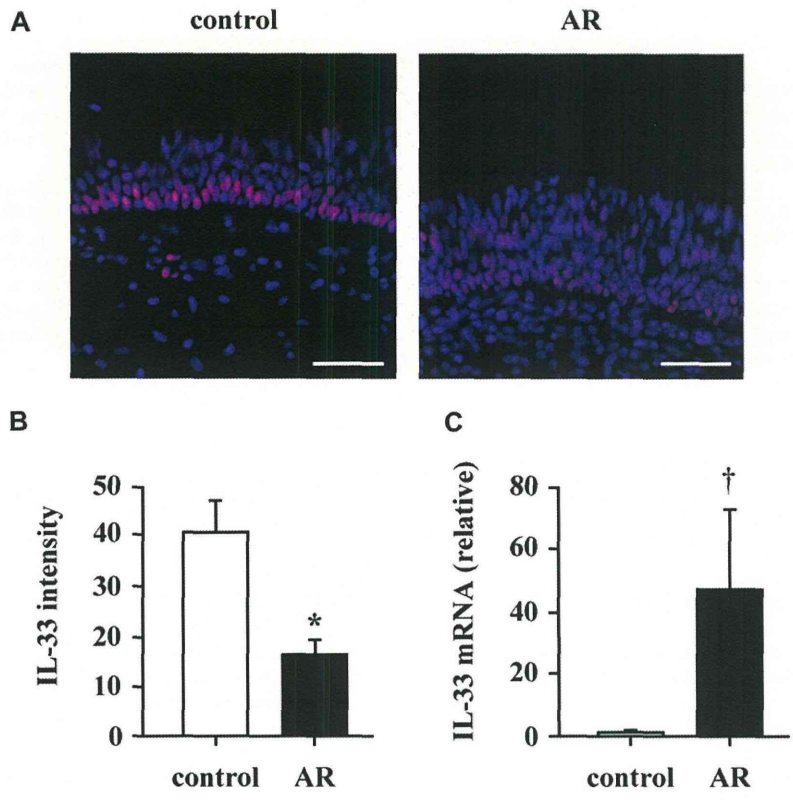


**FIG 7.** IL-33 is released from nasal epithelial cells in patients with AR. **A,** Immunofluorescence staining of nasal mucosal specimens from control subjects and patients with AR (see Table E1) stained for IL-33 *(red)* and 4′-6-diamidino-2-phenylindole dihydrochloride *(blue)*. *Bar* = 50 μm. Representative results from 5 healthy subjects and 10 patients with AR are shown. **B,** Quantitative image analysis of stained IL-33 in nasal mucosa. $*P < .05$. **C,** Relative IL-33 mRNA expression in nasal epithelial cells from control subjects and patients with AR (see Table E2), as determined by using real-time PCR. $\dagger P < .02$ compared with control subjects (Mann-Whitney *U* test).

nasal mucosa were somewhat decreased after nasal administration of ragweed pollen. To examine the number of basophils in the nasal mucosa of AR mice, we used TUG8, a recently established mAb that recognizes basophil-specific murine mast cell protease 8 (mMCP-8).[39] We found that the number of mucosal $TUG8^+$ basophils was significantly increased after nasal ragweed challenge; this suggests a recruitment of basophils from the circulating blood or memory basophils in the bone marrow. It has been reported in patients with seasonal AR after allergen challenge that the number of basophils increased significantly in the nasal mucosa, whereas the number of blood basophils decreased,[31,33] which supports an influx of basophils from the blood into the nasal mucosa. Importantly, the nasal accumulation of basophils was observed in ragweed-immunized mice but not in naive mice after provocation by ragweed pollen; this further substantiates that $Fc\varepsilon RI^+$ cells stimulated by IL-33 plus FcεRI cross-linkage are essential to the recruitment of basophils by producing chemoattractants for basophils.

Recently, 3 groups, including ours, have independently demonstrated that basophils are antigen-presenting cells that are necessary and sufficient for $T_H2$ priming both *in vitro* and *in vivo*.[28,53,54] In the present study, however, cervical lymph node cells from ragweed-challenged basophil-depleted mice produced almost the same amounts of IL-4 as control mice on stimulation *in vitro*, which suggests that basophils are not required for ragweed-induced $T_H2$ cell differentiation under these circumstances.

In summary, we established a novel ragweed-specific AR murine model, which could be very useful in the development of antiallergic drugs for AR. Also, we demonstrated that IL-33, promptly released from nasal epithelial cells in response to exposure to ragweed pollen, is essential for sneezing and the accumulation of eosinophils and basophils in the nasal mucosa by increasing histamine release and inducing production of chemoattractants from $Fc\varepsilon RI^+$ mast cells and basophils, respectively. IL-13 produced by IL-33–stimulated $T_H2$ cells, CTMCs, MMCs, and basophils induces goblet cell hyperplasia (see Fig E7 in this article's Online Repository at www.jacionline.org). This process, together with the contribution of IL-33 to stimulation of eosinophils,[19,20,55] basophils, and mast cells[16,18,51] to produce allergic inflammatory mediators, might lead to the recurrent seizures and irreversible mucosal hypertrophy seen in patients with AR. Thus IL-33 might present an important therapeutic target for the prevention of AR.

We thank Professor Hajime Karasuyama (Tokyo Medical and Dental University Graduate School, Tokyo, Japan) for providing an mAb specific for mMCP-8 and Ms Yuuko Taki for her assistance.

**Clinical implications: The discovery of ragweed pollen–driven endogenous IL-33 as a critical factor for the development of early- and late-phase responses in patients with AR might create a new therapeutic strategy for AR.**