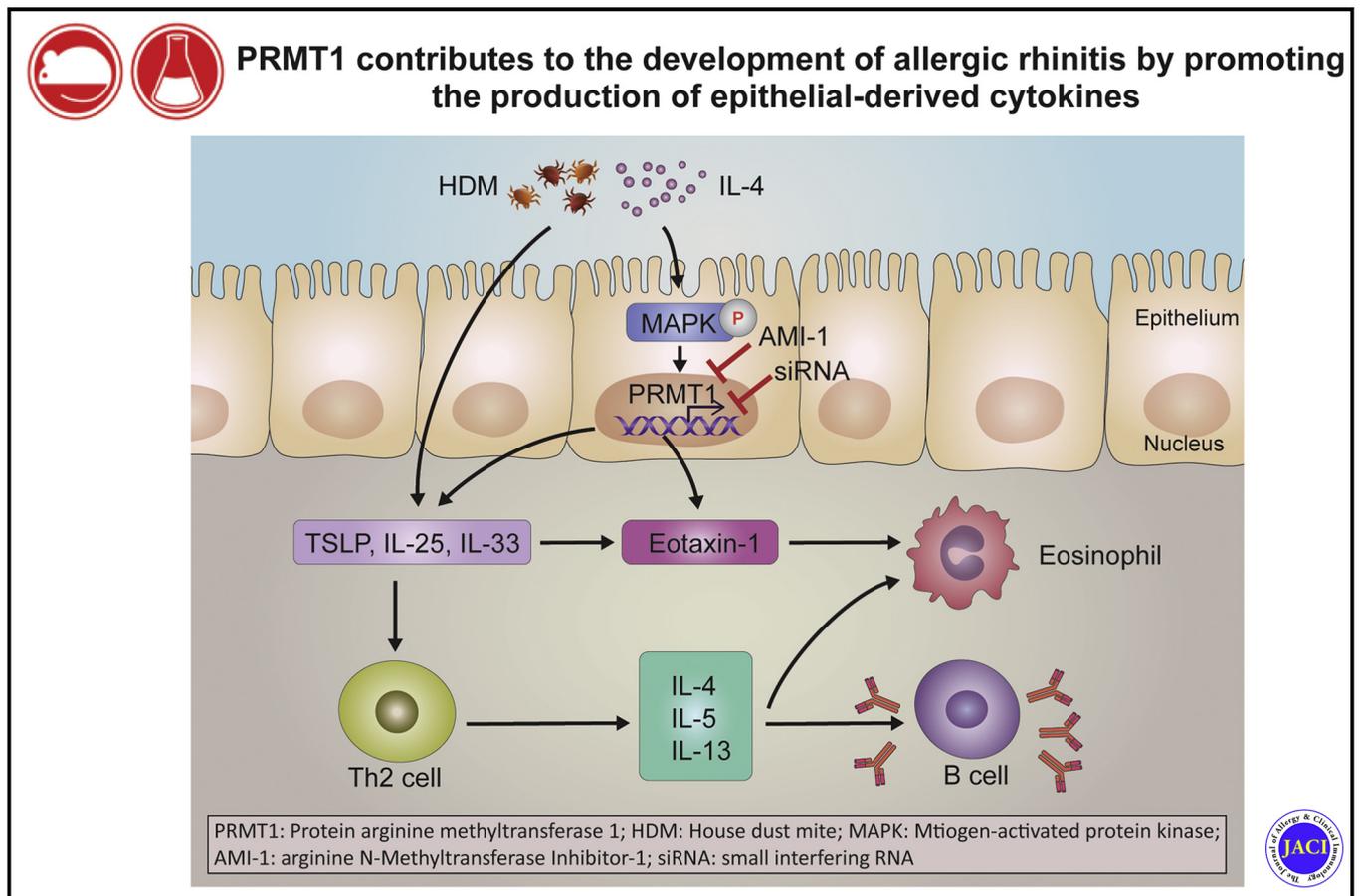


# Protein arginine methyltransferase 1 contributes to the development of allergic rhinitis by promoting the production of epithelial-derived cytokines



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## GRAPHICAL ABSTRACT



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**Background:** Arginine methylation is a posttranslational modification mediated by protein arginine methyltransferases (PRMTs). Although previous studies have shown that PRMT1 contributes to the severity of allergic airway inflammation or asthma, the underlying mechanism is poorly understood.

**Objective:** This study aimed to explore the role of PRMT1 and its relevant mechanism in the development of allergic rhinitis (AR).

**Methods:** The expression levels of PRMTs and cytokines were determined by RT-PCR, and the localization of PRMT1 was determined by immunohistochemistry and confocal microscopy. The levels of house dust mite (HDM)-specific immunoglobulins in serum and of cytokines in nasal lavage fluids were determined by ELISA. PRMT1 inhibition was achieved by siRNA and treatment with the pan PRMT inhibitor arginine *N*-methyltransferase inhibitor-1.

**Results:** PRMT1 expression was significantly increased in the nasal mucosa of patients and mice with AR. The degree of eosinophilic infiltration in the nasal mucosa was reduced in *PRMT1*<sup>+/-</sup> AR mice compared with wild-type mice. PRMT1 haploinsufficiency reduced the levels of HDM-specific immunoglobulins in serum and those of T<sub>H</sub>2 (IL-4, IL-5, and IL-13) and epithelial (thymic stromal lymphopoietin [TSLP], IL-25, and IL-33) cytokines in the nasal lavage fluids of AR mice. In nasal epithelial cells, HDM and IL-4 cooperate to enhance PRMT1 expression through a mitogen-activated protein kinase-dependent pathway. In addition, PRMT1 was essential for the production of TSLP, IL-25, and IL-33 in response to HDM and IL-4. Arginine *N*-methyltransferase inhibitor-1 treatment alleviated AR in the mouse model.

**Conclusions:** PRMT1 plays an important role in AR development by regulating epithelial-derived cytokine production and might be a new therapeutic target for AR. (*J Allergy Clin Immunol* 2021;147:1720-31.)

**Key words:** *PRMT1*, allergic rhinitis (AR), epithelial cytokines, MAPKs, house dust mite (HDM)

Allergic rhinitis (AR) is an upper airway disease caused by the IgE-mediated immune response of the nasal mucosa to environmental allergen exposure.<sup>1</sup> This disease affects 10% to 40% of the population worldwide, and its prevalence has increased rapidly over the last few decades.<sup>2</sup> AR can lead to nasal symptoms, such as sneezing, itching, runny nose, rhinorrhea, nasal congestion, and watery eyes, which can have significantly negative effects on quality of life and daily function and cause sleep disturbances and emotional problems.<sup>3</sup> Despite this high incidence and risk, the current therapies for AR are inadequate for some patients: (1) who have an eosinophil-dominant allergic nasal polyp or rhinitis,<sup>4</sup> (2) who have a high level of serum IgE or blood eosinophils,<sup>5</sup> or (3) who are inevitably exposed to high amounts of causal allergens during a season or at a working place, and no appropriate remedy or medicine is available.

Protein arginine methyltransferases (PRMTs) mediate the arginine methylation of proteins, which is a novel posttranslational modification.<sup>6,7</sup> This process contributes to multiple cellular events, including transcriptional regulation, signal transduction, chromatin structure, and DNA damage repair.<sup>8,9</sup> Based on the end products, PRMTs are classified into several families. Type I PRMTs include PRMT1, 2, 3, 4, 6, and 8, which are involved in the production of asymmetric dimethylarginine

#### Abbreviations used

ADMA:	Asymmetric dimethylarginine
AIPI:	Antigen-induced pulmonary inflammation
AMI-1:	Arginine <i>N</i> -methyltransferase inhibitor-1
AR:	Allergic rhinitis
COPD:	Chronic obstructive pulmonary disease
COX2:	Cyclooxygenase-2
HDM:	House dust mite
MAPK:	Mitogen-activated protein kinase
NAL:	Nasal lavage
NF-κB:	Nuclear factor kappa B
PRMT:	Protein arginine methyltransferase
<i>PRMT1</i> <sup>+/-</sup> :	PRMT1-haploinsufficient
TSLP:	Thymic stromal lymphopoietin
WT:	Wild-type

(ADMA). In contrast, type II PRMTs, such as PRMT5 and 9, generate symmetric dimethylarginine.<sup>10</sup> Among these, PRMT1 produces 90% of the generated ADMA.<sup>11</sup> DNA methylation and histone alterations play critical roles in cancer development, and PRMT1 is overexpressed in various human cancers.<sup>12</sup> In addition, arginine methylation by PRMT1 plays an important role in lung diseases, such as pulmonary fibrosis and chronic obstructive pulmonary disease (COPD), as well as lung cancer.<sup>13</sup> However, the role of PRMTs in the development of allergic diseases is limited.

Some studies have shown that PRMT1 contributes to antigen-induced pulmonary inflammation and allergic asthma.<sup>14-16</sup> PRMT1-3 expression in the lungs is upregulated in E3 rats with antigen-induced pulmonary inflammation (AIPI), and IL-4 leads to increased expression of PRMT1 in lung epithelial cells at an early stage of inflammation.<sup>16</sup> In contrast, at the chronic phase of AIPI, PRMT1 expression is upregulated mainly in fibroblasts and smooth muscle layers but not in airway epithelial cells.<sup>15</sup> TGF-β is responsible for the upregulation of PRMT1 and cyclooxygenase-2 in fibroblasts, and the blockage of PRMTs using arginine *N*-methyltransferase inhibitor-1 (AMI-1), a pan PRMT inhibitor, reduces cyclooxygenase-2 expression and asthmatic indexes in chronic AIPI-induced rats.<sup>15</sup> In addition, PRMT1 is constitutively and highly expressed in asthmatic airway smooth muscle cells, and this upregulation contributes to tissue remodeling by regulating cell proliferation and extracellular matrix production.<sup>14</sup> These findings demonstrate an association between arginine methylation and the development of allergic diseases, but the expression of PRMTs in the nasal mucosa and the role of PRMTs in AR development have not been studied. In the present study, we found that PRMT1 expression is upregulated in the nasal mucosa of patients with AR and a mouse model of AR. PRMT1 deficiency or inhibition alleviates allergic inflammation in the nasal mucosa of mice. In addition, house dust mite (HDM) and IL-4 synergistically induce PRMT1 expression in nasal epithelial cells and thereby regulate the production of thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, which are cytokines critical for T<sub>H</sub>2-cell differentiation.

## METHODS

Wild-type (WT) C57BL/6 mice and PRMT1-haploinsufficient (*PRMT1*<sup>+/-</sup>) mice had AR induced using HDM as depicted in Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). The gene expression of PRMTs was analyzed by RT-PCR in human and mouse nasal tissue. The

localization of PRMT1 was determined by immunohistochemistry and confocal microscopy. The degree of mucus secretion was examined by Alcian blue/periodic acid-Schiff staining, and the degree of eosinophil infiltration was assessed by Sirius red staining as previously.<sup>17</sup> The antigen-specific immunoglobulin levels in serum and cytokine levels in nasal lavage fluids or cell culture supernatants were measured by ELISA. In RPMI 2650 cells, the level of PRMT1 was determined by Western blotting and immunofluorescence. PRMT1 inhibition was achieved by siRNA and treatment with the pan PRMT inhibitor AMI-1. The complete and detailed methods are available in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

## RESULTS

### Expression of PRMT1 is upregulated in the nasal mucosa of patients with AR and AR mouse models

We first compared the gene expression of PRMTs in the nasal mucosa of non-AR controls and patients with AR. Among type I PRMTs, the PRMT1 and PRMT4 gene expression levels were significantly upregulated in the nasal mucosa of patients with AR compared with that of the control group (Fig 1, A and C), whereas neither PRMT3 nor PRMT5 showed significant differences in gene expression (Fig 1, B and D).

We further examined the expression of PRMTs in the nasal mucosa of HDM-induced AR mice (Fig E1). The gene expression of PRMT1 was significantly increased in the nasal mucosa of HDM-induced AR mice compared with that of the control mice (Fig 2, A). Consistent with the results obtained with the human samples, PRMT3 and PRMT5 expression in the nasal mucosa was comparable between the control and AR-induced mice (Fig 2, B and D). The mean expression level of PRMT4 was slightly higher in the AR-induced mice, but no significant difference in its expression level was found between the control and AR-induced mice (Fig 2, C). An immunohistochemistry analysis was also performed to demonstrate the presence and localization of PRMT1 in nasal tissues of AR-induced mice (Fig 2, E). When reacted with isotype IgG, no positive signal was found in the nasal tissues of mice with AR (Fig 2, E). PRMT1 was moderately expressed in the nuclei of nasal epithelial and stromal cells in PBS-treated or HDM-immunized mice (Fig 2, E and F), whereas very weak expression of PRMT1 was found in the cytoplasm of these cells (Fig 2, E). However, strong and moderate PRMT1 expression was observed in the nucleus and cytoplasm, respectively, of nasal epithelial cells of HDM-challenged AR mice (Fig 2, E and F). In Western blot analysis, PRMT1 protein was strongly detected in nasal mucosa of AR mice, but not in control mice (Fig 2, G and H). These results show that PRMT1 expression is upregulated in nasal tissues with AR, which indicates that PRMT1 might play a role in the development of AR.

### PRMT1 deficiency suppresses the development of AR in HDM-challenged mice

*PRMT1*<sup>+/-</sup> mice were used to determine the role of PRMT1 in the development of AR *in vivo* because mice with a homozygous allele of hypomorphic PRMT1 exhibit embryonic lethality.<sup>18</sup> Lower PRMT1 gene expression was detected in the nasal tissue of *PRMT1*<sup>+/-</sup> mice compared with that of WT mice (see Fig E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The analysis of Alcian blue/periodic acid-Schiff-stained sections revealed increased numbers of mucus-secreting cells in the nasal mucosa of HDM-challenged WT mice, whereas lower numbers were found in *PRMT1*<sup>+/-</sup> mice (Fig 3, A and B). In addition, Sirius

red staining revealed that HDM challenge led to massive eosinophil infiltration in the nasal mucosa of WT mice, whereas a lower eosinophilic infiltration score was given to *PRMT1*<sup>+/-</sup> mice (Fig 3, A and C).

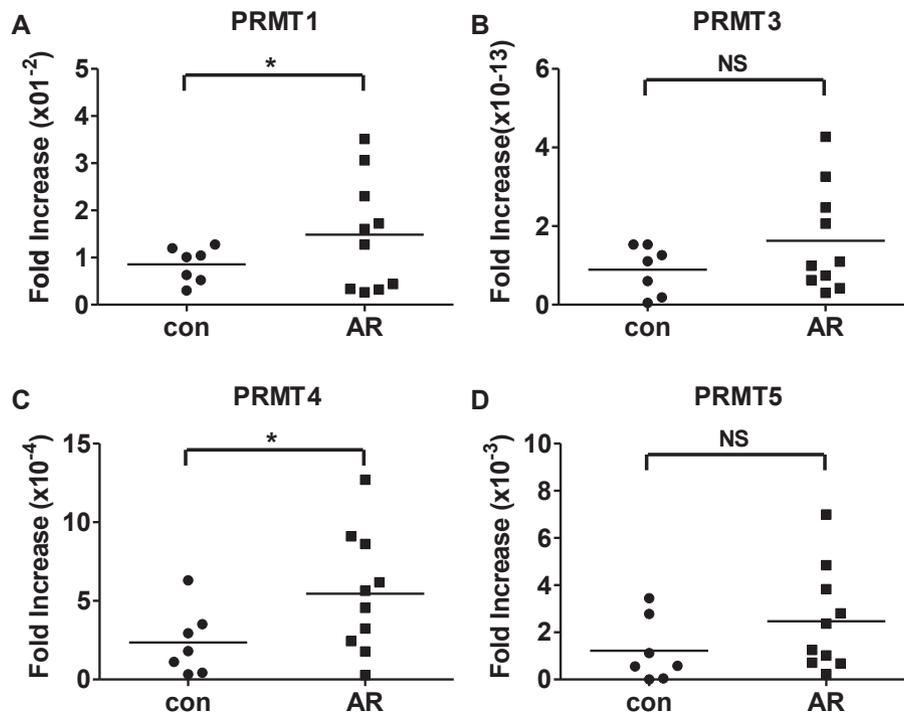
IgE is a critical factor for the development of allergic diseases because it promotes the production of inflammatory mediators such as histamine, prostaglandins, and cytokines in mast cells.<sup>19</sup> Therefore, we examined the involvement of PRMT1 in the production of HDM-specific antibodies, including IgE. The serum level of HDM-specific IgE after HDM challenge was significantly higher in WT mice than in *PRMT1*<sup>+/-</sup> mice (Fig 3, D). Moreover, the levels of the IgM, IgG, and IgG subclasses (IgG<sub>1</sub>, IgG<sub>2c</sub>, and IgG<sub>2b</sub>) specific to HDM were also higher in WT mice than in *PRMT1*<sup>+/-</sup> mice (Fig 3, E-I). These findings indicate that PRMT1 contributes to the development of AR by promoting antigen-specific IgE production.

### PRMT1 regulates T<sub>H</sub>2 cytokine production in nasal lavage fluids of mice with AR

Because T<sub>H</sub>2 cytokines, such as IL-4, IL-5, and IL-13, are responsible for IgE production by B cells, the activation and recruitment of eosinophils, and mucus production,<sup>20</sup> we further investigated whether PRMT1 contributes to the production of cytokines. HDM challenge increased the production of IL-4, IL-5, and IL-13 in nasal lavage (NAL) fluids, and the levels in *PRMT1*<sup>+/-</sup> mice were significantly lower than those in WT mice (Fig 4, A-C). The levels of IFN- $\gamma$  and IL-17 in NAL fluids were also increased by HDM challenge, but comparable levels were detected in WT and *PRMT1*<sup>+/-</sup> mice (see Fig E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Because the epithelial production of TSLP, IL-25, and IL-33 is essential for T<sub>H</sub>2 or type 2 innate lymphoid cell differentiation,<sup>21</sup> the cytokine levels were also determined. Substantial levels of TSLP, IL-25, and IL-33 were detected in NAL fluids of HDM-challenged WT mice, and the levels in *PRMT1*<sup>+/-</sup> mice were significantly lower (Fig 4, D-F). It is likely that PRMT1 participates in the allergen-mediated production of T<sub>H</sub>2 cytokines through upregulation of the epithelial production of TSLP, IL-25, and IL-33.

### HDM and rIL-4 synergistically enhance PRMT1 expression in human nasal epithelial cells through mitogen-activated protein kinase-dependent pathways

HDM extract can activate nasal epithelial cells and enhance the expression of various genes.<sup>22-24</sup> In addition, IL-4 produced by T<sub>H</sub>2 cells upregulates PRMT1 gene expression in respiratory epithelial cells via a positive feedback loop.<sup>16</sup> To determine a molecular mechanism underlying the enhancement of PRMT1 expression in a human nasal epithelial cell line, RPMI 2650 cells were treated with HDM and/or rIL-4. Treatment with HDM or rIL-4 alone slightly increased the gene and protein expression of PRMT1 in RPMI 2650 cells, and their combination markedly enhanced its expression compared with that in cells treated with HDM or rIL-4 alone (Fig 5, A-C). We further determined the localization of PRMT1 in HDM/rIL-4-treated cells because its localization differs depending on the disease and cell type.<sup>25-28</sup> Confocal microscopy showed that the nuclear expression of PRMT1 in the cells was enhanced by HDM/rIL-4 treatment (Fig 5, D and E). In addition, HDM/rIL-4 treatment enhanced



**FIG 1.** Gene expression of PRMTs in the nasal mucosa of healthy patients and patients with AR. **A-D**, The gene expression of PRMT1, PRMT3, PRMT4, and PRMT5 in the nasal mucosa of non-AR control and patients with AR was measured by RT-PCR. *con*, Control; *NS*, nonsignificant. The horizontal bars indicate the means. \**P* < .05.

ADMA formation in nasal epithelial cells (Fig 5, F). Because mitogen-activated protein kinases (MAPKs) are known to be involved in the regulation of PRMT1 expression,<sup>14,29,30</sup> we assessed whether HDM/rIL-4 treatment influences MAPK activation in RPMI 2650 cells. HDM and rIL-4 cotreatment slightly enhanced the phosphorylation of ERK and p38 starting at 30 minutes and more strongly promoted JNK phosphorylation starting at 15 minutes (Fig 5, G). PD98059 (an ERK inhibitor) and SB203580 (a p38 inhibitor) suppressed the HDM-and-rIL-4-mediated increase in PRMT1 expression (Fig 5, H and I). The JNK inhibitor SP600125 also downregulated the expression of PRMT1 to the level found in untreated cells (Fig 5, H and I). The HDM/rIL-4-induced nuclear expression of PRMT1 was also reduced by MAPK inhibitors (Fig 5, J and K). These findings indicate that HDM and rIL-4 might cooperate to increase PRMT1 expression and promote its nuclear localization through the MAPK pathway, particularly JNK-dominant signaling, in nasal epithelial cells.

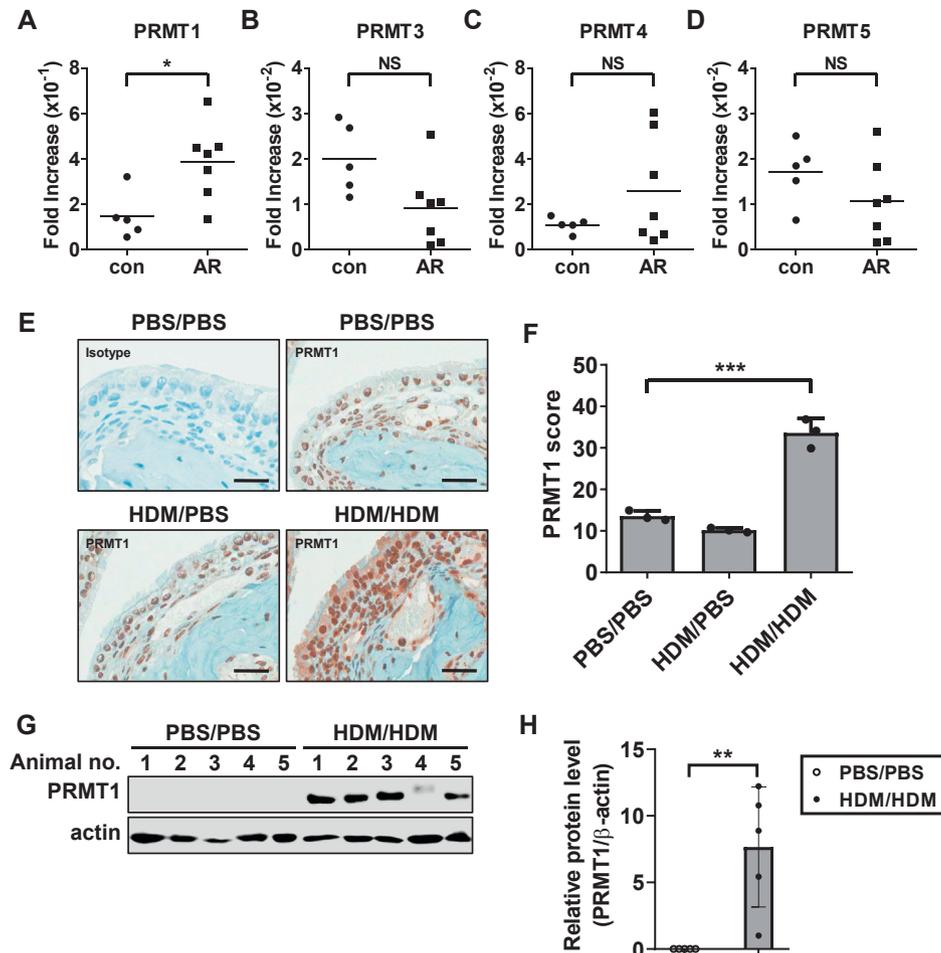
### PRMT1 mediates the expression of TSLP, IL-25, and IL-33 in nasal epithelial cells in response to HDM and rIL-4

PRMT1 regulates gene transcription and protein activity by catalyzing the methylation of histones and cellular proteins.<sup>10</sup> To determine whether PRMT1 regulates the expression of TSLP, IL-25, and IL-33 in nasal epithelial cells, PRMT1 expression in RPMI 2650 cells was inhibited by siRNA, and the cells were subsequently treated with HDM and rIL-4. siRNA successfully reduced the gene and protein expression of PRMT1 in the cells

(see Fig E4, A and B, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Cotreatment with HDM and rIL-4 enhanced the gene expression of TSLP, IL-25, IL-33, and eotaxin-1 in RPMI 2650 cells, and this enhancement was not detected in the cells in which PRMT1 was inhibited by siRNA (Fig 6, A, and Fig E4, C). In contrast, PRMT1 inhibition by siRNA did not influence gene expression of MUC5AC, MUC5B, CXCL1, and CCL2 upregulated by cotreatment with HDM and rIL-4 (Fig E4, D-G). To confirm the occurrence of this phenomenon under more physiological conditions, human primary nasal epithelial cells were prepared under air-liquid interface culture conditions. Consistently, the PRMT1 expression was upregulated in human primary nasal epithelial cells after treatment with HDM and rIL-4 (Fig 6, B). AMI-1 treatment reduced the HDM-and-rIL-4-induced enhancement of TSLP, IL-25, IL-33, and eotaxin-1 gene expression in human primary nasal epithelial cells (Fig 6, C, and Fig E4, D). During culture for 3 days, the production of TSLP in the culture supernatant was increased by HDM and rIL-4 treatment, and this increase was partially inhibited by AMI-1 (Fig 6, D). IL-25 and IL-33 protein expression was undetectable in this system (data not shown). These findings indicate that PRMT1 might contribute to the development of AR by regulating the production of TSLP, IL-25, and IL-33 in nasal epithelial cells in response to allergen and IL-4.

### AMI-1 treatment alleviates HDM-induced AR in mice

We subsequently sought to determine whether the pharmacological inhibition of PRMT1 alleviates AR development *in vivo*.



**FIG 2.** PRMT1 expression in the nasal mucosa of HDM-induced AR mice. **A-D**, The gene expression of PRMT1, 3, 4, and 5 in the nasal mucosa of control and AR-induced mice was measured by RT-PCR. **E**, Immunohistochemistry was performed to determine the cellular localization of PRMT1 expression in the nasal mucosa of mice. **F** and **H**, The density of nucleus-expressed PRMT1 was measured using ImageJ program, and the results are expressed as the means  $\pm$  SDs. **G**, The PRMT1 protein levels in the nasal tissues of mice ( $n = 5$ ) with or without AR were determined by Western blotting. **H**, Relative protein levels were obtained by dividing the density of PRMT1 by that of  $\beta$ -actin and the results are also expressed as the means  $\pm$  SDs. Bar = 20  $\mu$ m. *con*, Control; *NS*, nonsignificant. \* $P < .05$ . \*\* $P < .01$ . \*\*\* $P < .001$ .

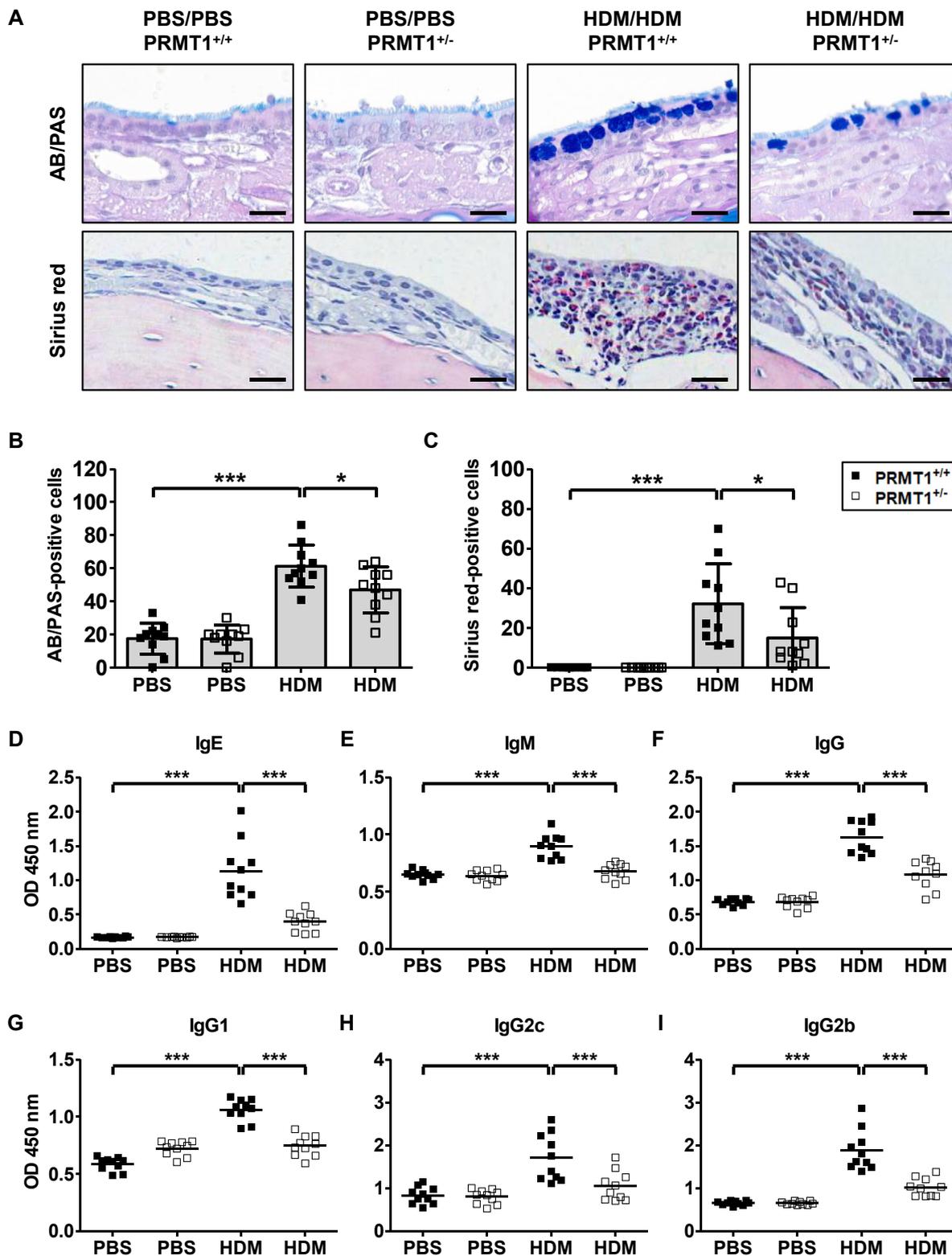
The pan PRMT inhibitor AMI-1 was intraperitoneally given to the mice before and after HDM challenge in accordance to the experimental schedule depicted in Fig 7, A. As shown in Fig 7, B to D, AMI-1 treatment reduced the infiltration of mucus-secreting cells and eosinophils in the nasal mucosa of AR-induced mice. AMI-1 treatment also decreased the production of immunoglobulins tested, except IgG<sub>2c</sub>, in serum (Fig 7, E; see Fig E5 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). In NAL fluids, the production of IL-4 was also decreased by AMI-1 treatment (Fig 7, F). The mean levels of IL-5 and IL-13 in NAL fluids were also decreased by treatment with AMI-1, but this decrease was not significant (Fig 7, F). The levels of TSLP, IL-25, and IL-33 in NAL fluids were also lower in the AMI-treated mice (Fig 7, G).

## DISCUSSION

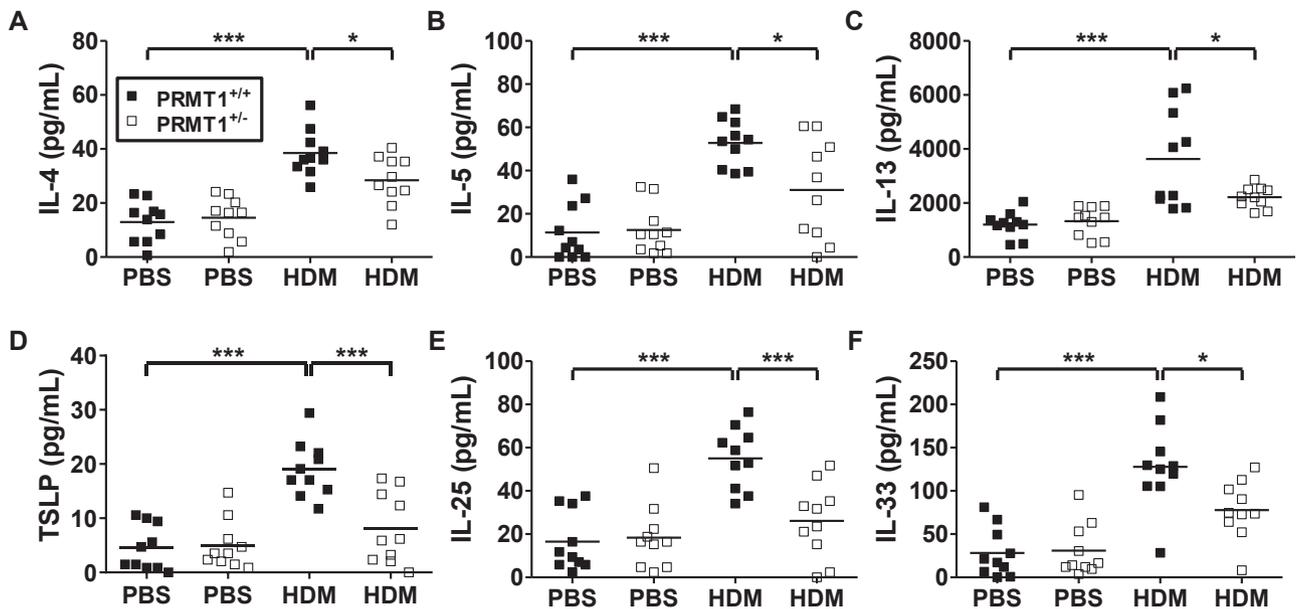
Although PRMT1 is involved in the pathophysiology of various diseases, including chronic pulmonary inflammation, the role of PRMT1 in the development of AR has not been

studied. In the present study, we revealed that PRMT1 expression was upregulated in the nasal mucosa of patients and mice with AR and that PRMT1 deficiency or inhibition reduced eosinophil infiltration in the nasal mucosa and the production of antigen-specific immunoglobulins, including IgE, and T<sub>H</sub>2 cytokines, such as IL-4, IL-5, and IL-13, in serum. PRMT1 was also essential for TSLP, IL-25, and IL-33 production in nasal epithelial cells in response to HDM and IL-4.

Aberrant expression of PRMTs, mainly their overexpression, appears to be associated with the development of several diseases. PRMT1 overexpression has been detected in various cancers, such as lung, breast, prostate, colon, and bladder cancers.<sup>8</sup> During osteoclastogenesis, RANKL upregulates PRMT1 expression in bone marrow cells, which is critical for nuclear factor kappa B (NF- $\kappa$ B) activation and osteoclastogenesis-related gene expression.<sup>29</sup> Moreover, an association between increased PRMT1 expression and allergic airway inflammation has been found. E3 rats with AIP1 exhibited increased PRMT1, 2, and 3 expression and lower PRMT4 expression in the lungs compared with control



**FIG 3.** Goblet cell metaplasia, eosinophil infiltration, and serum levels of antigen-specific immunoglobulins in WT and *PRMT1*<sup>-/-</sup> mice with HDM-induced AR. **A**, Goblet cell metaplasia and eosinophil infiltration were evaluated in AB/PAS- or Sirius red-stained sections of WT and *PRMT1*<sup>-/-</sup> mice with and without AR, respectively. **B** and **C**, AB/PAS- and Sirius red-positive cell counts were obtained as described in the Methods section, and the results are expressed as the means  $\pm$  SDs. **D-I**, Levels of IgE, IgM, IgG, and IgG subtypes specific to HDM in the mouse serum were measured by ELISA. AB/PAS, Alcian blue/periodic acid-Schiff. The horizontal bars indicate the means. Bar = 20  $\mu$ m. \**P* < .05. \*\*\**P* < .001. Bar = 20  $\mu$ m.



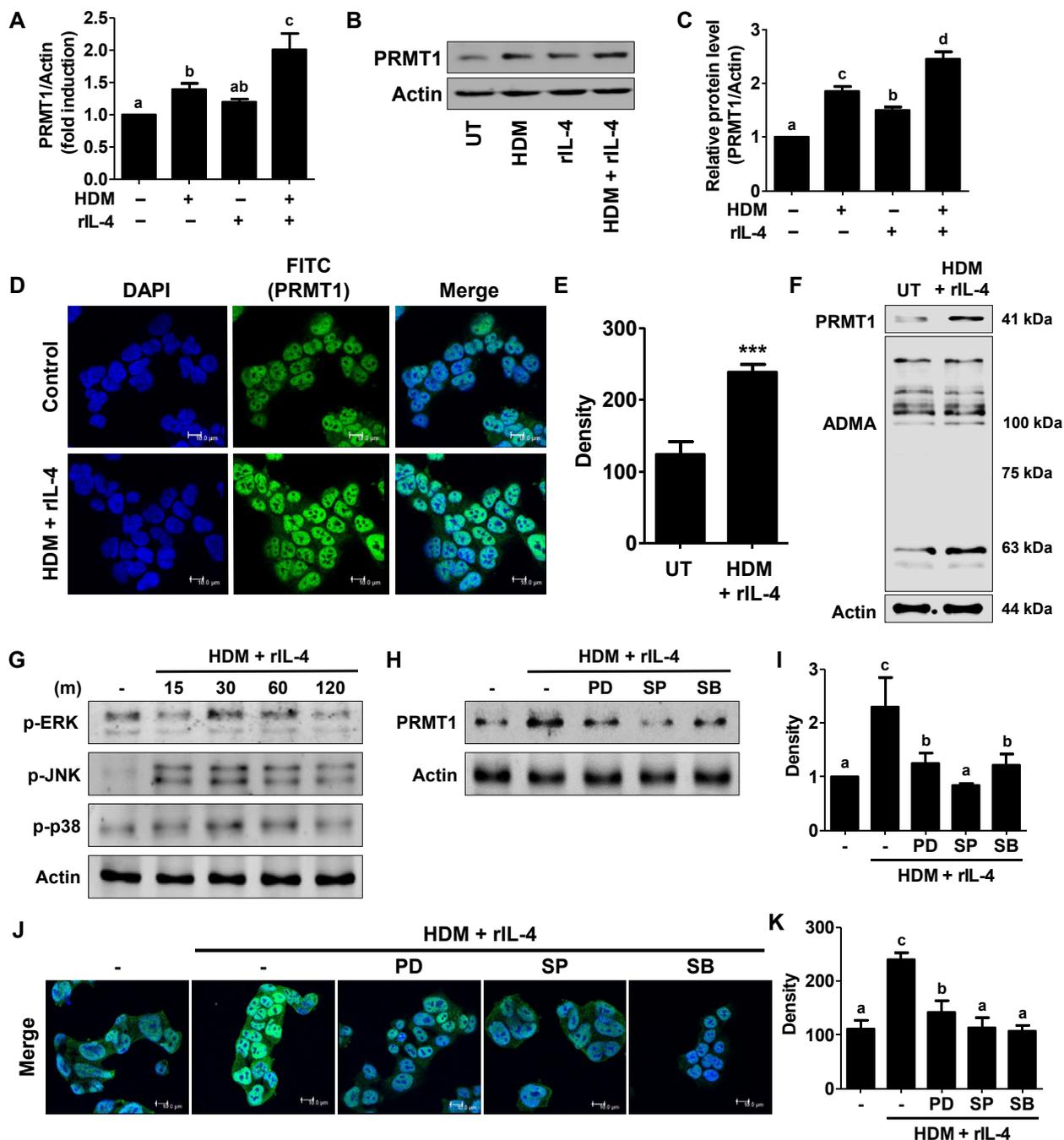
**FIG 4.**  $T_H2$ - and epithelial-derived cytokine production in NAL fluids of AR-induced WT and *PRMT1*<sup>+/-</sup> mice. **A-F**, NAL fluids were obtained by flushing the nasal cavity with 700  $\mu$ L of PBS. **A-C**, The levels of  $T_H2$  cytokines, including IL-4, IL-5, and IL-13, were measured by ELISA. **D-F**, The levels of epithelial-derived cytokines, including TSLP, IL-25, and IL-33, were measured by ELISA. The horizontal bars indicate the means. \* $P < .05$ . \*\*\* $P < .001$ .

mice.<sup>16</sup> The expression of PRMT5 and PRMT6 appears to be unaffected.<sup>16</sup> In the present study, PRMT1 and PRMT4 expression was upregulated in the nasal mucosa of patients with AR. In addition, PRMT1 was significantly increased in the nasal mucosa of HDM-induced AR mice compared with that of the control mice. Although no significant difference in PRMT4 expression was found between the 2 groups, its mean level was slightly higher in the nasal mucosa of AR mice. This finding is inconsistent with the results of a previous study conducted by Sun et al,<sup>16</sup> which found lower expression of PRMT4 in the lung tissue of E3 rats with AIPI. Whether this discrepancy is due to differences in tissue origin remains unclear. In the present study, we investigated the role of PRMT1 in AR development because PRMT1 is overexpressed in the nasal mucosa of both humans and mice with AR and is responsible for more than 90% of ADMA formation.<sup>11</sup> Whether PRMT4 contributes to AR development should thus be clarified.

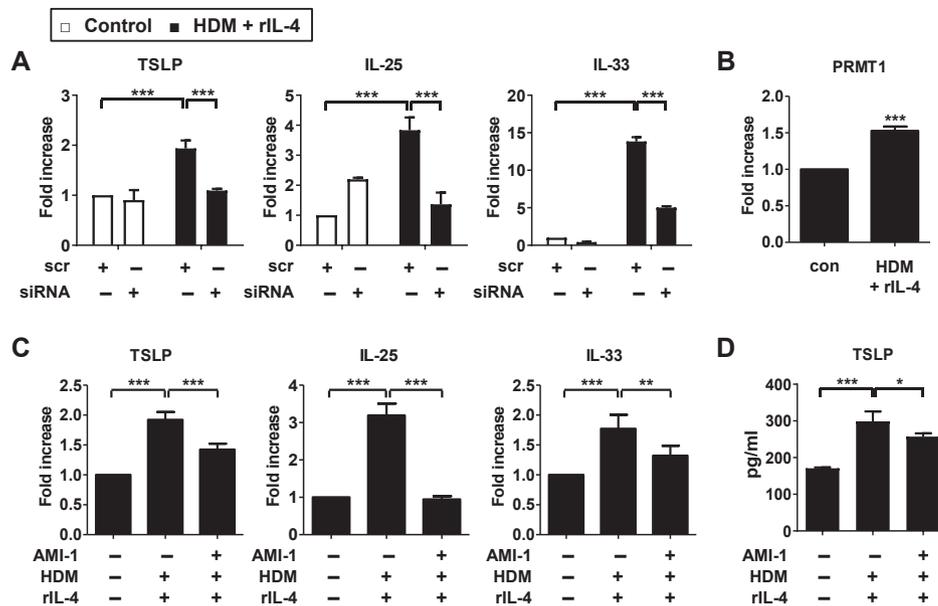
A previous study showed that PRMT1 is mostly expressed in epithelial cells at the acute phase of AIPI, whereas its expression is strongly detected in fibroblasts or smooth muscle cells, but not epithelial cells, at the chronic phase.<sup>15</sup> In addition, PRMT1 is more highly expressed in airway smooth muscle cells from patients with asthma compared with healthy controls,<sup>14</sup> which suggests that PRMT1 can be expressed in various cell types in the respiratory tract. In this study, strong expression of PRMT1 was mostly observed in the nucleus of nasal epithelial cells in both humans and mice with AR, although its nuclear expression was also observed in inflammatory and stromal cells. Moreover, the experimental schedule of the HDM-induced AR mouse model is similar to that of the acute AIPI model.<sup>15</sup> These findings suggest that PRMT1 might play an important role in nasal epithelial cells during AR development, at least at the acute phase.

In our data, 4 of 10 patients in the AR group had a low level of PRMT1, but there was no significant difference in eosinophil count or total IgE level among patients with AR. Interestingly, we realized that the 3 of them were nonsmoker and another patient was a short-term (<10 pack-years) smoker, whereas most patients with AR with a high level of PRMT1 were more than 10 pack-years of ex-smoker or current long-term smokers. In airway epithelial cells, only a small overlap in DNA methylation is observed with whole blood and magnitude of changes is about 10 times larger than what was seen in the peripheral blood,<sup>31-33</sup> suggesting specific targets in airway epithelial cells. In addition, COPD is usually related to tobacco smoking and develops in mid to later life, and arginine methylation by PRMT1 was suggested to play an essential role in COPD.<sup>13</sup> Therefore, our data suggest that smoking history seems to be a factor influencing PRMT1 levels.

PRMT1 can be localized in both the nucleus and cytoplasm and shows high mobility between both regions depending on the methylation status of the substrate proteins in several cells.<sup>34</sup> This shuttling controls the processes of signal transduction, subcellular protein trafficking, and gene expression.<sup>34,35</sup> In normal and cancerous tissues of the breast and colon, PRMT1 staining is mostly detected in the cytoplasm and is rarely detected in the nucleus.<sup>26,27</sup> The researchers of these previous studies claim that the enzyme is likely localized in the cytoplasm because methylation might occur during or just after translation.<sup>26,27</sup> In contrast, PRMT1 expression is mostly found in the nucleus of gastric cancer tissues, which is associated with poor diagnosis.<sup>25</sup> In lung fibroblasts isolated from patients with idiopathic pulmonary fibrosis, PRMT1 is predominantly localized in the nucleus and moderately expressed in the cytoplasm.<sup>28</sup> In the present study, we found strong PRMT1 expression in the nuclei of nasal epithelial cells of patients and mice with AR, although weak to moderate PRMT1 expression was still observed in the cytoplasm.



**FIG 5.** PRMT1 expression and localization in nasal epithelial cells in response to HDM and IL-4. **A-J**, RPMI 2650 cells were treated with HDM (100  $\mu$ g/mL) and/or rIL-4 (10 ng/mL). **A**, The gene expression of PRMT1 after 8 hours of treatment was analyzed by RT-PCR. **B** and **C**, The PRMT1 protein levels after 24 hours of treatment were confirmed by Western blotting and quantified. **D** and **E**, The localization of PRMT1 expression was identified and quantified by immunofluorescent confocal microscopic analysis. **F**, The ADMA formation after 72 hours of treatment was examined by Western blotting. **G**, The expression of phosphorylated forms of ERK, JNK, and p38 was detected by Western blotting. **H-K**, Cells were pretreated with MAPK inhibitors, such as PD98059, SP600125, and SB203580, for 2 hours and subsequently incubated with HDM and rIL-4 for an additional 24 hours. **H** and **I**, The PRMT1 protein levels were confirmed by Western blotting and quantified. **J** and **K**, The PRMT1 localization was determined and quantified by confocal microscopic analysis. DAPI, 4'-6-Diamidino-2-phenylindole, dihydrochloride; FITC, fluorescein isothiocyanate. The results are expressed as the means  $\pm$  SDs. Values with different letters are significantly ( $P < .05$ ) different, as determined by ANOVA with Duncan's multiple range test. \*\*\* $P < .001$ .



**FIG 6.** PRMT1 inhibition reduces TSLP, IL-25, and IL-33 expression in nasal epithelial cells in response to HDM and IL-4. RPMI 2650 cells (A) and A549 cells (B) were transfected with scramble or PRMT1 siRNA and then treated with HDM (100  $\mu$ g/mL) and rIL-4 (10 ng/mL) for 12 hours. A and B, The gene expression of TSLP, IL-25, IL-33, and PRMT1 was measured by RT-PCR. C and D, Human primary nasal epithelial cells were pre-treated with AMI-1 (20  $\mu$ g/mL) for 1 hour and then treated with HDM and rIL-4 for an additional 12 or 72 hours. C, The gene expression of TSLP, IL-25, and IL-33 was measured by RT-PCR. D, The TSLP levels in culture supernatants were detected by ELISA. The results are expressed as the means  $\pm$  SDs. \* $P$  < .05. \*\* $P$  < .01. \*\*\* $P$  < .001.

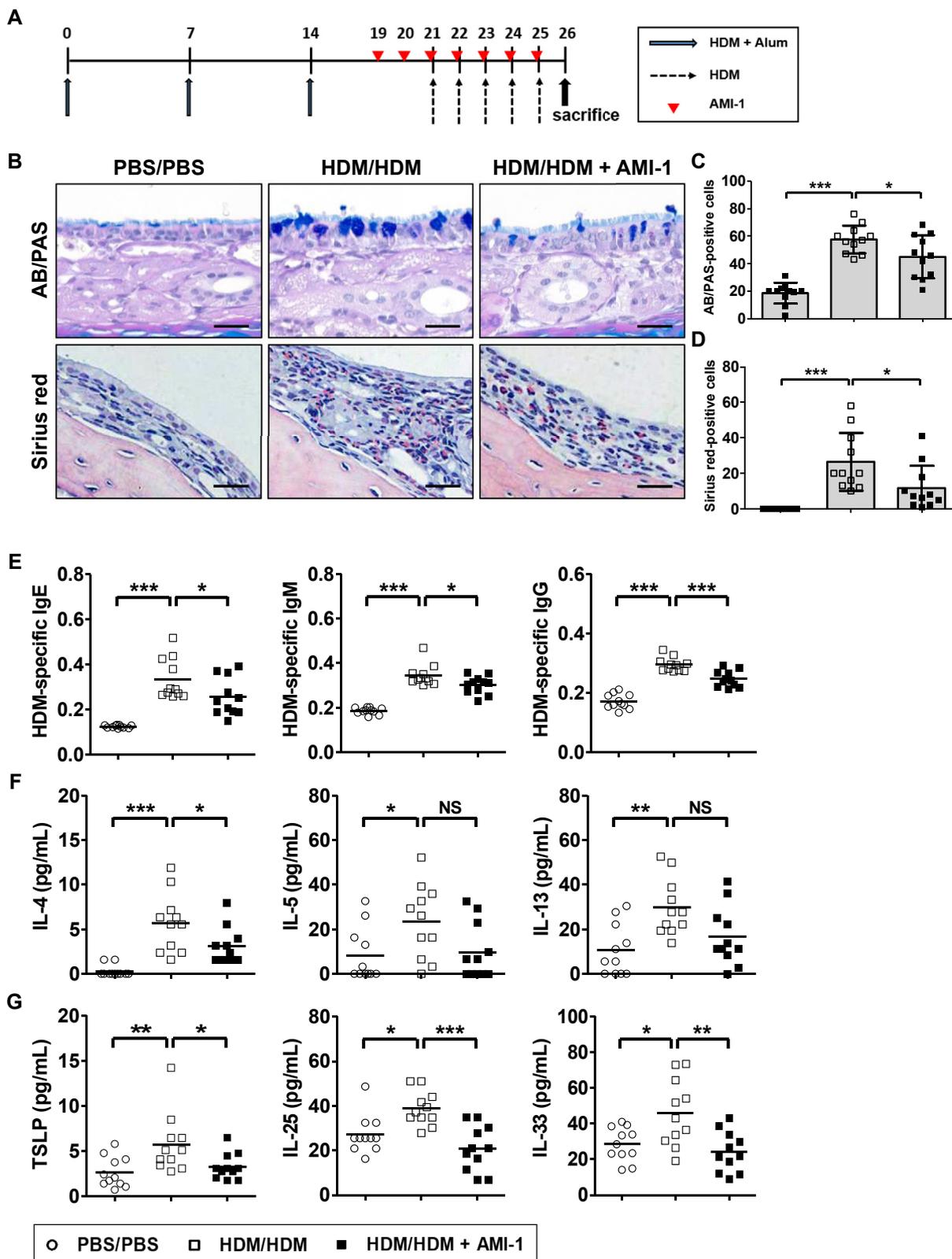
Moreover, confocal microscopy observations showed that the nuclear expression of PRMT1 in RPMI 2650 cells was enhanced in response to HDM and rIL-4 and thus supported this hypothesis. The identities of the genes or proteins that enhance PRMT1 regulation in the nucleus of nasal epithelial cells remain to be elucidated.

Previous studies have shown that IL-4 promotes PRMT1 expression in lung fibroblasts and airway epithelial cells.<sup>16,28</sup> In the present study, IL-4 also upregulated the gene and protein expression of PRMT1 in nasal epithelial cells. Interestingly, HDM synergized with IL-4 to enhance PRMT1 expression and its nuclear localization in the cells, which suggests that allergens might cooperate with IL-4 to regulate PRMT1 expression. MAPK family members, such as ERK, JNK, and p38, play a central role in mediating multiple signaling pathways involved in inflammation, immunity, and cell survival. MAPKs have been shown to regulate PRMT1 expression under various experimental conditions. Lim et al<sup>30</sup> showed that hypoxia-induced PRMT1 expression is downregulated by inhibitors of p38 and JNK, but not ERK, in human lung epithelial cells. Platelet-derived growth factor induces ERK phosphorylation in airway smooth muscle cells and thereby regulates PRMT1 expression.<sup>14</sup> In addition, RANKL treatment promotes PRMT1 expression in bone marrow cells through a JNK-dependent pathway, which is essential for osteoclastogenesis.<sup>29</sup> In this study, we showed that cotreatment with HDM and rIL-4 induces the phosphorylation of all MAPKs (p38, ERK, and JNK) with different kinetics and to different degrees in RPMI 2650 cells. Each inhibitor can reduce HDM/rIL-4-induced PRMT1 expression, and in the presence of a JNK inhibitor, the expression level reached almost basal levels, which suggests that the MAPK pathway with JNK dominance

might be essential for regulating PRMT1 expression in nasal epithelial cells.

Protein arginine methylation might play unique roles in humoral immune responses, including B-cell proliferation, differentiation, and survival. Hata and Mizuguchi<sup>36</sup> detected reduced proliferation of B cells and decreased differentiation of these cells into IgG<sub>1</sub>-secreting cells in the presence of arginine methyltransferase inhibitor after stimulation with LPS/IL-4/CD40-L.<sup>36</sup> Mouse models with PRMT1-null B cells obtained with the Cre-LoxP system showed impaired B-cell development and function in a T-cell-independent but not T-cell-dependent manner.<sup>37</sup> Furthermore, immunization with protein plus adjuvant or influenza virus infection resulted in impaired germinal center-related humoral immune responses, including the generation of antibody-secreting cells, in these mouse models.<sup>38</sup> A defect in PRMT1 activity is reportedly associated with the regulation of FOXO1 or PI3K,<sup>39</sup> which is essential for isotype switching and differentiation into antibody-secreting cells and subsequent germinal center formation.<sup>40</sup> In the present study, the serum levels of all antigen-specific immunoglobulins, including IgM, IgG and its subtypes, and IgE, were significantly higher in the WT mice than in the *PRMT1*<sup>+/-</sup> mice. Whether this impaired antigen-specific immunoglobulin production is due to defects in PRMT1-mediated B-cell differentiation and isotype switching and whether impaired PRMT1 function in B cells influences HDM-induced AR development and severity in mice remain unclear, and these topics should be further investigated using a mouse model with tissue-specific depletion of PRMT1.

The airway epithelium, which responds to external environmental factors, links the innate and adaptive immune systems



**FIG 7.** AMI-1 administration attenuates AR severity in mice. **A**, Schematic representation of the experimental protocol. **B**, Representative images of Sirius red- and H&E-stained nasal tissue sections. **C** and **D**, AB/PAS- and Sirius red-positive cell counts were obtained as described in the Methods section, and the results are expressed as the means  $\pm$  SDs. **E**, The serum levels of HDM-specific IgE, IgM, and IgG were measured by ELISA. **F**, The levels of  $T_H2$  cytokines, including IL-4, IL-5, and IL-13, were measured by ELISA. **G**, The levels of epithelial-derived cytokines, including TSLP, IL-25, and IL-33, were measured by ELISA. AB/PAS, Alcian blue/periodic acid-Schiff; H&E, hematoxylin & eosin. The horizontal bars indicate the means. Bar = 20  $\mu$ m. \* $P$  < .05. \*\*\* $P$  < .001.

through the release of cytokines and chemokines.<sup>41</sup> TSLP, IL-25, and IL-33 are representative cytokines of epithelial-derived alarmins.<sup>41</sup> A recent study showed that IL-25, IL-33, and TSLP are mainly produced and secreted by epithelial cells in response to cell damage, pathogen recognition, or allergen exposure.<sup>41,42</sup> These epithelial-derived cytokines mediate various pathophysiological responses of allergic reactions by initiating T<sub>H</sub>2 cell and type 2 innate lymphoid cell responses, which results in the production of cytokines such as IL-4, IL-5, and IL-13.<sup>41-45</sup> Our results showed that HDM and IL-4 upregulated TSLP, IL-25, and IL-33 expression in nasal epithelial cells in a PRMT1-dependent manner. In fact, the cytokine levels in NAL fluids were reduced in the PRMT1<sup>+/-</sup> or AMI-1-treated mice with HDM-induced AR, and the levels of T<sub>H</sub>2 cytokines (IL-4, IL-5, and IL-13) in NAL fluids and IgE in serum were also lower in these mice. Accordingly, PRMT1 likely contributes to AR development by initiating the epithelial production of TSLP, IL-25, and IL-33 in response to allergen and IL-4 and subsequently inducing T<sub>H</sub>2 immunity.

PRMT1 is an asymmetric arginine methyltransferase and the H4R3me2as modification catalyzed by PRMT1 is a typical marker of active chromatin. TSLP, IL-25, and IL-33 are epithelial-derived cytokines, which are involved in allergic diseases by inducing type 2 responses. In this study, we demonstrated that PRMT1 mediates the expression of TSLP, IL-25, and IL-33 genes in nasal epithelial cells under HDM and rIL-4 treatment. Because the PRMT1-mediated histone modification of H4R3me2as is associated with transcriptional activation of various genes,<sup>46</sup> it is of great interest to understand the mechanism by which PRMT1 regulates the epithelial-derived cytokine expressions. Musiani et al<sup>47</sup> reported that PRMT1 phosphorylated by DNA-PK is recruited to chromatin and then methylates H4R3 at the promoter of the senescence-associated secretory phenotype gene, whose expression is transcriptionally regulated by NF- $\kappa$ B.<sup>47</sup> In addition, PRMT1 can directly methylate the RelA subunit of NF- $\kappa$ B to form a cellular complex with it. This process leads to transcriptional regulation of target genes by manipulating the affinity between NF- $\kappa$ B and the target genes in response to TNF- $\alpha$ .<sup>48</sup> It is also known that the gene expression of TSLP, IL-25, and IL-33 is regulated by the activation of NF- $\kappa$ B.<sup>49-53</sup> Further studies are needed to determine whether PRMT1 regulates gene expression of the epithelial-derived cytokines via direct modification of histone at the target promoter regions or regulation of NF- $\kappa$ B activity.

## Conclusions

PRMT1 is highly expressed in nasal epithelial cells, particularly in the nucleus, of hosts with AR. Allergen and T<sub>H</sub>2 cytokines, such as IL-4, appear to cooperate to upregulate PRMT1 expression. PRMT1 contributes to AR development by regulating the production of epithelial cytokines, such as TSLP, IL-25, and IL-33, in response to allergens and IL-4, which results in the promotion of differentiation into T<sub>H</sub>2 cells, IgE production, and eosinophil recruitment. The administration of the pan PRMT inhibitor AMI-1 alleviates the severity of HDM-induced AR in mice, which suggests that PRMT1 might be a new therapeutic target for AR. A new PRMT1-specific inhibitor that can be clinically applicable for patients with AR thus needs to be developed.

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## Key messages

- PRMT1 expression is upregulated in the nasal mucosa of patients and mice with AR.
- HDM and IL-4 cooperate to induce PRMT1 expression via MAPKs' signaling.
- PRMT1 inhibition alleviates the disease severity in AR mice.

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