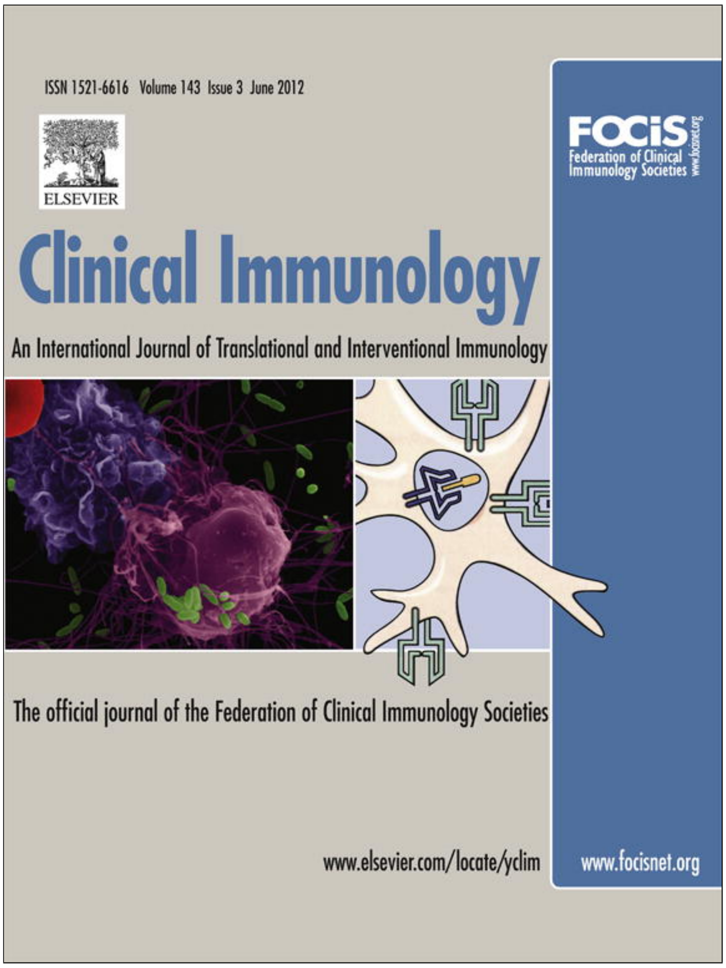
ISSN 1521-6616 Volume 143 Issue 3 June 2012
ELSEVIER
Clinical Immunology
An International Journal of Translational and Interventional Immunology
FOCIS
Federation of Clinical Immunology Societies
The official journal of the Federation of Clinical Immunology Societies
www.elsevier.com/locate/yclim
www.focisnet.org

Clinical Immunology (2012) **143**, 256–265

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

AIMP1 deficiency enhances airway hyperreactivity in mice via increased T_H2 immune responses

Hye-Jin Hong [a], Eugene Kim [a], Mi Young Jung [a], Sunghoon Kim [b], Tae Sung Kim [a,*]

[a] *Division of Life Sciences, School of Life Sciences and Biotechnology, Korea University, Seoul 136–713, Republic of Korea*
[b] *Center of Medicinal Protein Network and Systems Biology, College of Pharmacy, Seoul National University, Seoul 151–742, Republic of Korea*

Received 19 January 2012; accepted with revision 21 February 2012
Available online 3 March 2012

KEYWORDS
AIMP1;
Asthma;
CD4;
Dendritic cell;
T_H2 response

Abstract Aminoacyl tRNA synthetase complex-interacting multicomplex protein 1 (AIMP1) is known as a novel cytokine carrying out a variety of biological activities, including angiogenesis and wound repair. In our previous reports AIMP1 was demonstrated to induce T_H1 polarization. However, the effects of AIMP1 deficiency in T_H1 or T_H2 immune disorders remain unclear. In this study, we characterized phenotypes of AIMP1-deficient mice and investigated the role of AIMP1 in T_H2-biased airway hyperreactivity. Clinical signs of allergic airway inflammation were assessed in AIMP1-deficient mice and the effects of AIMP1 deficiency on production of T_H2 cytokines were evaluated in T cells using AIMP1-specific siRNA. Additionally, the enhanced pause values and histologic analysis were assessed in mice receiving AIMP1-deficient $CD4^+$ T cells with OVA challenge. Clinical signs of spontaneous airway inflammation were noted in AIMP1-deficienct mice. AIMP1-deficient mice showed strongly increased Penh values in response to methacholine without any allergen exposure. Adoptive transfer of AIMP1-deficient $CD4^+$ T cells to OVA-sensitized C57BL/6 mice exacerbated OVA-induced airway inflammation and increased infiltration of inflammatory cells into the lung. Furthermore, lung DCs in AIMP1-deficient mice showed increased expression of surface molecules, and IL-12p40 level in sera significantly decreased in AIMP1-deficient mice compared to that of wild type mice. These results strongly indicate that AIMP1 plays a role in negatively regulating T_H2 responses *in vivo*, and AIMP1 can be employed as a novel therapeutic agent against T_H2-biased diseases, particularly asthma.

Abbreviations AIMP1, Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1; BALF, Bronchoalveolar lavage fluid; AHR, Acute hyperreactivity; BMDC, Bone marrow-derived dendritic cell; Penh, Enhanced pause

* Corresponding author at: School of Life Sciences and Biotechnology, Korea University, 5-ga, Anam-dong, Seongbuk-gu, Seoul 136–713, Republic of Korea. Fax: +82 2 3290 3921.
E-mail address: tskim@korea.ac.kr (T.S. Kim).

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doi:10.1016/j.clim.2012.02.004

1. Introduction

Allergic asthma is one of the most common T_H2-biased immune disorders among public health problems; it is characterized by airway eosinophilia, high level of IgE, excessive production of T_H2 cytokines and airway remodeling [1,2]. IL-4, IL-5 and IL-13 are produced in large quantities by activated T_H2 lymphocytes, and IL-5 in particular is a key cytokine in asthma pathogenesis [3]. Glucocorticoids and cyclosporine are commonly employed to prevent or treat allergic disease [4], but corticosteroids have been shown to be rather inefficient and to induce severe side effects [5]. In those reasons, researchers have attempted to develop novel therapeutic modalities involving the application of endogenous factors such as genes, cytokine-derived peptides, or transcription factors.

$CD4^+$ T helper (Th) cells are regarded as crucial immune players in adaptive immunity. T_H2 cells, a Th cell subset, activate inflammation that make antibodies and produce cytokines such as IL-4, IL-5 and IL-13, and recruit eosinophils and basophils to inflammatory regions in allergic disease via chemokine production [6,7]. Therefore, it is regarded as a useful treatment for asthma to suppress the function of activated $CD4^+$ T cells. Many of the T_H2 cell effector pathways of allergic inflammation are involved with dendritic cells (DCs) which strongly lead to T cell-mediated adaptive immune responses [8]. Particularly in asthma, DCs are at the nexus of innate and adaptive immunity in lung, recognizing inhaled allergens and initializing adaptive T_H2 responses [9]. Recently, several unique molecules altering DC functions have been identified and suggested as therapeutic targets via inhibiting biological potentials or stimulating immunoregulatory properties of DCs [10,11]. It is important to understand how DCs control T_H2 cell immunity or how to control DCs to conquer T_H2 immune disorders, particularly asthma.

AIMP1 (aminoacyl tRNA synthetase complex-interacting complex 1) is 35 kDa polypeptide consisting of 312 amino acids, a nonenzymatic auxiliary factor of macromolecular aminoacyl tRNA synthetase [12]. AIMP1 itself is secreted by various stimulations such as TNF-α and heat shock proteins, and is known to carry out a variety of biological activities, including angiogenesis and wound repair [13–15]. According to our previous reports, recombinant AIMP1 induced the activation of macrophages and bone marrow-derived dendritic cell (BMDC), resulting in T_H1 differentiation *in vitro*, suggesting that AIMP1-stimulated DC might function as a cell vaccine in immunotherapy against cancer [16,17]. Although

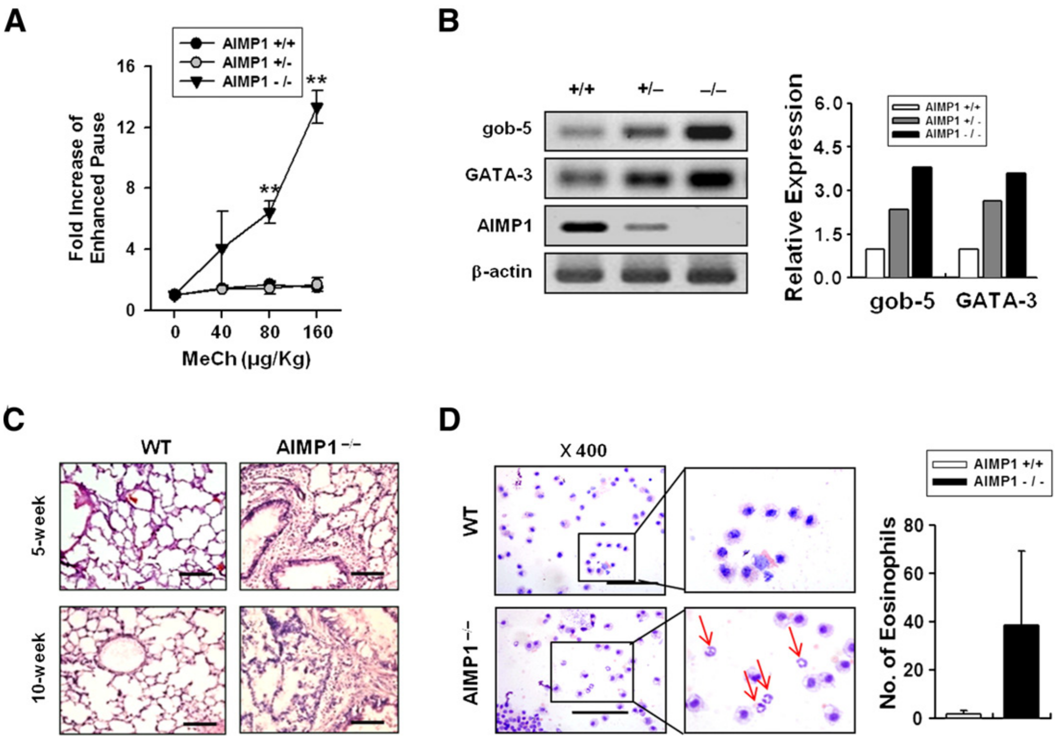

Figure 1 Clinical signs of spontaneous airway inflammation in $AIMP1^{-/-}$ mice.(A) Acute hyperresponsiveness was measured after administration with increasing doses of methacholine. The results were expressed as fold increase of enhanced pause relative to baseline values. Data represent the means ± SEM (ANOVA, $^{**}p<0.005$, *vs* $AIMP1^{+/+}$). (B) mRNA expressions in lung were detected by RT-PCR analysis using lung homogenates of 8-week old mice. Band intensity was determined densitometrically and expressed as relative intensity to the corresponding control of wild type mice. Data are representative of three independent experiments. (C) Lungs of 5- and 10-week old mice were prepared for histology. Lungs were stained with hematoxylin and eosin following 0.8 μm cryosection. Histological analysis was conducted by light microscopy (bars: 100 μm). (D) Bronchoalveolar lavage fluids were assessed by Giemsa staining. Each cell-type in BALFs was counted by independent investigators under light microscope (bars: 50 μm, arrows: eosinophils). Original magnification of the right is x400, and black-borded boxes were further magnified. Data are representative of three independent experiments. Data in the right panel represents the means ± SEM.

recent studies have indicated various immunological functions of AIMP1 and the correlation of lung immunity, it remains to be clearly elucidated the effects of AIMP1 on CD4$^+$ T cell-mediated immune disorder, particularly T_H2 type allergic airway inflammation.

In this study, we demonstrated that AIMP1-deficiency induced spontaneous airway inflammation. AIMP1-deficient CD4$^+$ T cells secreted enhanced T_H2 cytokines and exacerbated OVA-induced airway inflammation. Furthermore, IL-12 production decreased under AIMP1-deficient conditions. These data indicate that AIMP1 may function as an endogenous negative regulator against T_H2 responses.

2. Materials and Methods

2.1. Mice

AIMP1-deficient mice (C57BL/6 background) were constructed as previously described, and the deficiency of an AIMP1 gene was confirmed before the experiments [18]. All animal experiments were conducted in accordance with the institutional animal care guidelines of Korea University (Approval No. KUIACUC-1/5/2009-2).

2.2. Preparation of lung and bronchoalveolar lavage fluids (BALFs)

Lungs were fixed in 4% paraformaldehyde and dehydrated with 40% sucrose. Lungs were stained with hematoxylin and eosin following 0.8 μm cryosectioning. BALFs were prepared by washing the bronchi and alveoli with PBS containing 0.1% FBS, and stained with Giemsa solution. Each cell-type in BALFs was counted by three independent investigators for the differential counts. Histology was examined by light microscope (Olympus IX71; Olympus, Tokyo, Japan) and visualized by RS Image software.

2.3. Analysis of cytokine production and immunoglobulins by ELISA

To evaluate antigen-specific cytokine production, freshly isolated peripheral lymph node cells from the OVA-immunized mice were restimulated with 100 μg/ml OVA. Cytokine levels in the supernatants were assessed via ELISA for IL-4, IL-5 (48 hr) and IFN-γ (72 hr) as previously described [19]. To measure immunoglobulin levels, sera were isolated from the blood, and immunoglobulins were measured via ELISA as previously described [20]. To measure cytokine production from CD4$^+$ T cells, freshly isolated CD4$^+$ T cells using anti-mouse

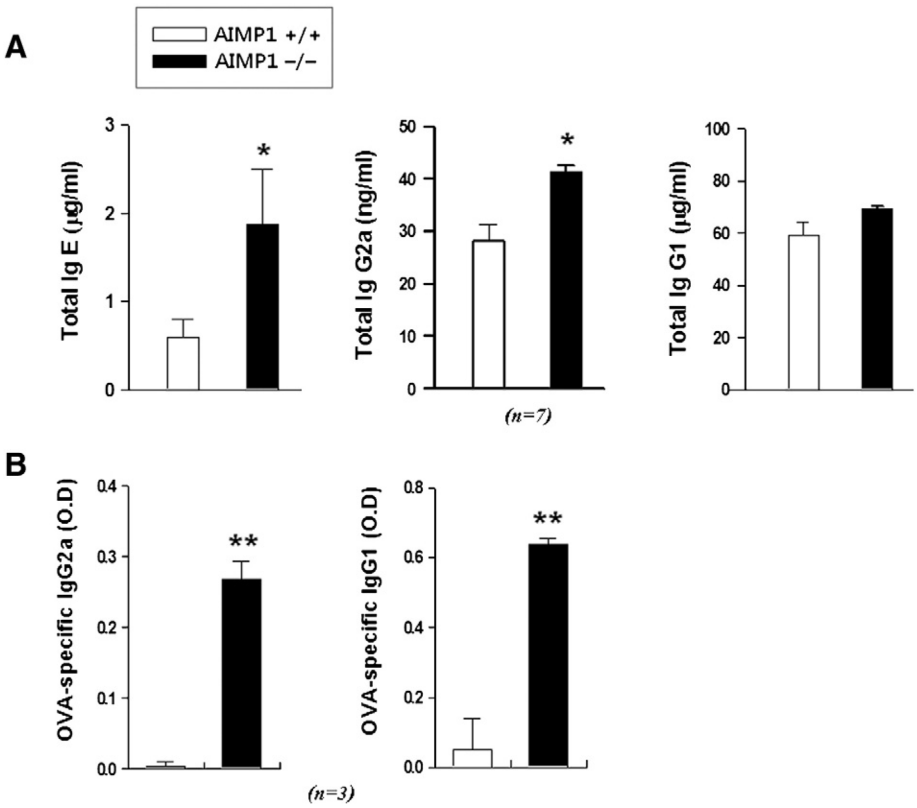

Figure 2 Increased levels of IgE, IgG1 and IgG2a by AIMP1 deficiency. (A) Sera were extracted from 7 to 8-week old mice, and the levels of total IgE, IgG2a and IgG1 were determined by ELISA. Data present the means ± SEM (*p<0.05, *vs* AIMP1$^{+/+}$). (B) Eight-week old mice were received 50 μg OVA in 2 mg alum, intraperitoneally, on day 0 and 7. Mice were sacrificed on day 14. OVA-specific immunoglobulin levels in sera were determined via OVA-specific ELISA. Data represent the $O.D_{490}$ ± SEM (*p <0.05, **p<0.005, *vs* AIMP1$^{+/+}$).

CD4 MicroBeads (Miltenyi Biotec, Paris, France) were stimulated for 48 hr with anti-CD3 and anti-CD28 antibodies. To measure production levels of cytokines from EL-4 cells, EL-4 cells were stimulated for 24 hr with 50 ng/ml PMA, 1 μM ionomycin and 500 μM cAMP. The levels of IL-4, IL-5, and IFN-γ in the culture supernatants were determined via ELISA.

2.4. Silencing endogenous AIMP1 by using small interfering RNA (siRNA)

EL-4 cells were cultured in RPMI containing 10 mM sodium pyruvate, 50 μM 2-ME, 10 mM HEPES, and 10% FBS, after which the cells were washed with Opti-MEM serum-reduced medium (Invitrogen, Carlsbad, CA) and seeded on 24-well culture plates (2.5×10^5 cells/well). The cells were transiently transfected with non-silencing scrambled siRNA duplex (Bioneer, Seoul, Korea) or three kinds of AIMP1-specific siRNA (Bioneer) in the presence of Lipofectamine2000 reagent (Invitrogen). After 4 hr, the culture supernatants were replaced with the fresh growth medium and the silencing effects of AIMP1 gene were determined via Western blot analysis and RT-PCR. The transfection efficiency was consistently > 95%, as determined by flow cytometric analysis.

2.5. Study protocol and analysis of OVA-induced airway inflammation

Mice intraperitoneally received 50 μg OVA/2 mg alum on day 0 and day 7. Aerosolized OVA was delivered to the mice on

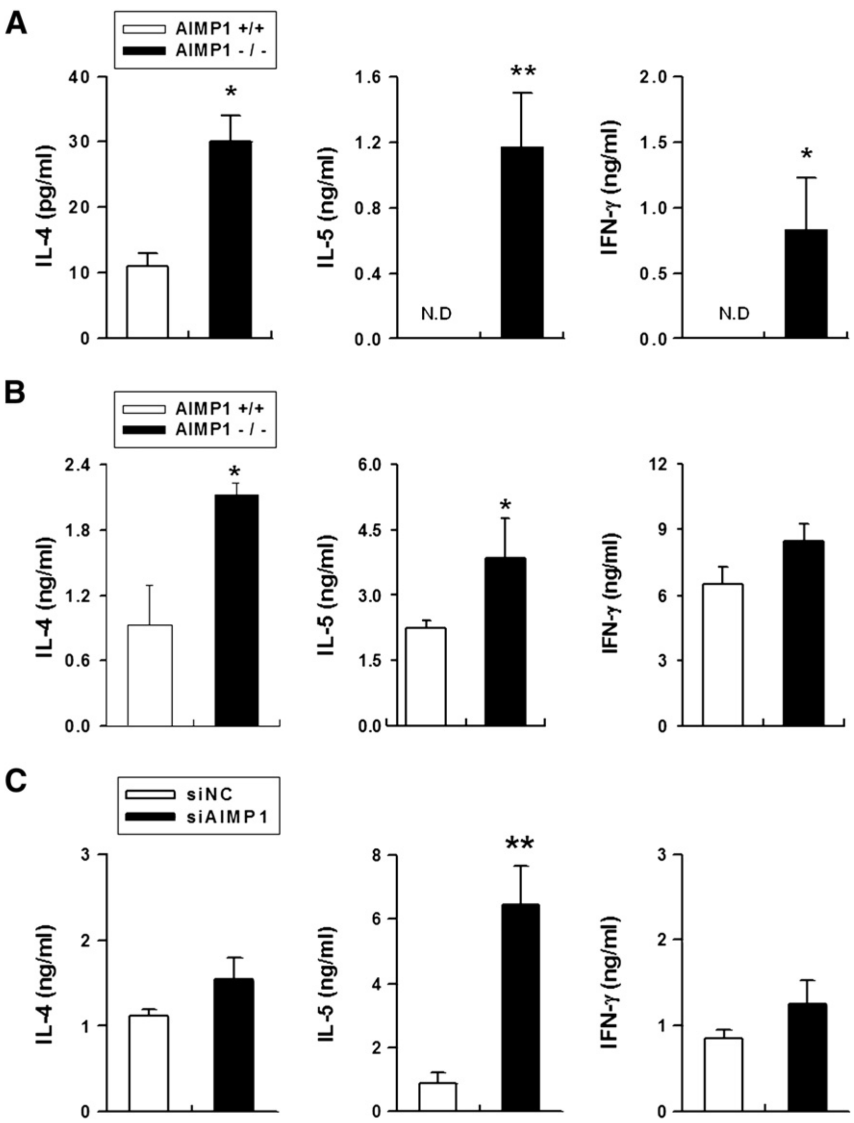

Figure 3 Increased levels of T_H2 cytokines by AIMP1 deficiency.(A) Eight-week old mice received 50 μg OVA in 2 mg alum subcutaneously on day 0 and 7, after which the mice were sacrificed on day 14. Single cell suspensions of draining lymph nodes were restimulated for 48 hr with 100 μg/ml OVA, and the supernatants were determined for production levels of IL-4, IL-5, and IFN-γ via ELISA. (B) $CD4^+$ T cells were isolated from peripheral lymph nodes and stimulated with anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml) for cytokine secretion. (C) EL-4 cells which received siAIMP1 or control siRNA were stimulated for 24 hr with PMA (50 ng/ml), ionomycin (1 μM) and cAMP (500 μM). Afterwards, the levels of IL-4, IL-5 and IFN-γ in the culture supernatants were determined via ELISA. Data represent the means ± SEM. *$p<0.05$, **$p<0.005$, *vs* $AIMP1^{+/+}$, N.D, not detected.

day 14–16 (3% OVA) and day 17 (10% OVA) for 20 min per day by Pulmo-Aid Nebulizer (Sunrise Medical, Longmont, CO). For the measurement of AHR in response to β-methacholine (Wako Pure chemical Ind., Osaka, Japan) in asthmatic mice, OCP-3000 (Allmedicus, Seoul, Korea) was used as a noninvasive whole body plethysmography. This animal study protocol was approved by the Korea University Institutional Animal Care & Use Committee.

2.6. Flow cytometric analysis

BMDCs or lung DCs were obtained as previously described, and washed with cold PBS containing 0.05% FBS and sodium azide [10]. The cells were incubated with FITC-conjugated CD11c (HL3), or with PE-conjugated mAbs including anti-I-A^b (AF-120.1), anti-CD40 (3/23), anti-CD86 (GL1), anti-CD80 (16-10A1) and anti- ICOSL (HK5.3). The stained cells were examined by FACS Calibur (BD Biosciences, San Diego, CA), and analyzed using Cell Quest Pro software (BD Biosciences).

2.7. Statistics

The data were expressed as the means ± standard error of the mean (SEM) (* $p<0.05$, ** $p<0.005$). Statistical differences between experimental and control groups were determined via Student *t*-test or one-way analysis of variance (ANOVA) followed by Dunnett's method (SPSS 12.0; Apache Software Foundation).

3. Results

3.1. AIMP1-deficiency induces spontaneous airway hyperreactivity in mice

As AIMP1 is localized throughout the lung [21] and regulates Th cell-mediated immune responses [16,17], AIMP1 may function in Th cell-mediated immune disorders occurring in the lungs, such as asthma. As a way of gaining a role of AIMP1 in T_H1 or T_H2 immune disorders, the effect of AIMP1 deficiency on airway hyperreactivity was investigated in AIMP1-deficient mice. The construction details of AIMP1$^{-/-}$ deficient mice were described previously [18]. Interestingly, AIMP1-deficient mice showed strongly increased Penh values in response to methacholine without any allergen exposure (Fig. 1A). mRNA expression of gob-5 which is related to mucus production, and GATA-3, a major transcription factor of T_H2 polarization, was spontaneously increased in AIMP1-deficient mice (Fig. 1B). Furthermore, we found that AIMP1-deficient mice evidenced more increased infiltrations of inflammatory cells around airways as they aged (Fig. 1C). Spontaneous eosinophil recruitment was observed in the BALFs of AIMP1-deficient mice (Fig. 1D). Total IgE, one of the clinical features of T_H2 immune disorders, was significantly high in the sera of AIMP1-deficient mice (Fig. 2A), in consistent with the previous report [18]. These results indicate that AIMP1-deficient mice seem to develop spontaneous airway hyperreactivity.

3.2. AIMP1-deficiency enhancesT_H2 immune responses

To determine whether AIMP1-deficiency influences antigen-specific responses, wild type and AIMP1-deficient mice were sensitized with 50 μg OVA via subcutaneous and intraperitoneal injections on day 0 and day 7, and OVA-specific immunoglobulins and cytokines were determined via ELISA. As shown in Fig. 2B, OVA-sensitization triggered a significant increase in levels of OVA-specific IgG1 and IgG2a in AIMP1-deficient mice, compared with those of wild type mice. OVA-specific IgE was not detected in all groups. Moreover, in OVA-sensitized AIMP1-deficient mice, the levels of IL-4, IL-5 and IFN-γ were significantly increased (Fig. 3A). Those results clearly show that AIMP1-deficiency enhanced OVA-specific T_H2 responses upon antigen sensitization. Interestingly, the proportion of $CD4^+$ T cells in periperal lymph nodes was significantly higher in AMP1-deficient mice than in the wild type (Table 1). Thus, we further investigated whether $CD4^+$ T cells are involved in the increased T_H2 cytokine production and spontaneous inflammation in AIMP1-deficient mice. $CD4^+$ T cells were isolated from naïve wild type C57BL/6 and AIMP1-deficient mice, followed by incubation for 48 hr with TCR stimulation. IL-4 and IL-5 levels significantly increased in AIMP1-deficient mice, compared with those of wild-type mice (Fig. 3B). To further substantiate that the increased levels of T_H2 cytokines were directly attributable to AIMP1-deficiency, we delivered AIMP1-specific siRNA into EL-4 T cells. The most efficient siRNA for AIMP1 knockdown was chosen based on the silencing efficiency of each siRNA as demonstrated by RT-PCR and Western blot analysis (Fig. S1). Production levels of T_H2 cytokines, particularly IL-5, significantly increased in the AIMP1-knockdowned EL-4 cells (Fig. 3C). Taken together, these results indicate that the AIMP1-deficiency may enhance the production of T_H2 cytokines from $CD4^+$ T cells causing spontaneous airway hyperreactivity in AIMP1-deficient mice.

Table 1 Proportion of various immune cells in the secondary lymphoid organs.

Organ	Cell-type	AIMP1$^{+/+}$	AIMP1$^{-/-}$
Spleen	$CD4^+$	20.07 ± 2.35	21.32 ± 2.68
	$CD8^+$	12.20 ± 1.46	12.86 ± 1.64
	$CD3^+$	41.66 ± 7.27	39.31 ± 7.21
	$B220^+$	50.88 ± 7.12	45.26 ± 7.46
	$NK1.1^+$	6.88 ± 2.38	7.30 ± 1.92
	$F4/80^+$	7.80 ± 4.65	8.78 ± 2.75
	$CD11c^+$	6.96 ± 4.97	5.55 ± 3.88
Peripheral lymph node	$CD4^+$	36.59 ± 1.64	43.18 ± 3.92*
	$CD8^+$	26.78 ± 2.32	25.30 ± 4.00
	$CD3^+$	78.17 ± 14.09	79.34 ± 8.72
	$B220^+$	31.92 ± 2.87	27.06 ± 4.80
	$NK1.1^+$	3.22 ± 1.83	4.52 ± 3.32
	$F4/80^+$	3.50 ± 1.53	3.92 ± 1.00
	$CD11c^+$	4.59 ± 1.59	3.81 ± 1.61

Proportion of each cell-type was evaluated in the secondary lymphoid organs of in AIMP1$^{+/+}$ and AIMP1$^{-/-}$ mice via flow cytometric analysis. The values represent the means ± SEM.
* *p* value <0.005, *vs* AIMP1$^{+/+}$.

3.3. AIMP1-deficient CD4$^+$ T cells exacerbate OVA-induced airway hyperreactivity

To more directly investigate whether the absence of AIMP1 in CD4$^+$ T cells induces T_H2 responses, we adoptively transferred AIMP1-deficient or wild-type CD4$^+$ T cells into the AHR murine model (Fig. 4A). Adoptive transfer of AIMP1-deficient CD4$^+$ T cells induced greatly increased AHR in response to methacholine compared with that of wild type CD4$^+$ T cells (Fig. 4B), and aggravated lung inflammation via the infiltration of large amounts of inflammatory cells at the peribronchial and perivenular regions in the lungs

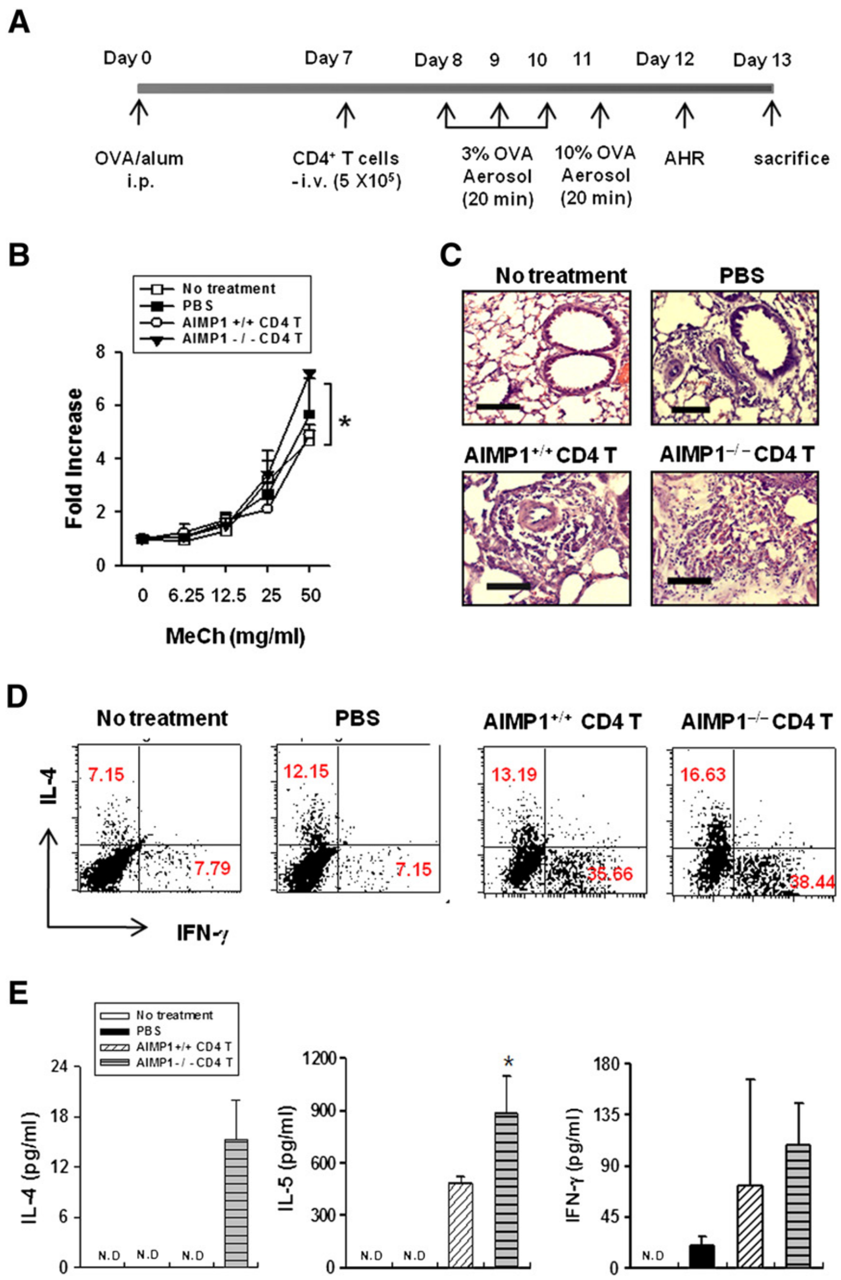

Figure 4 Enhanced AHR in mice receiving AIMP1-deficient CD4$^+$ T cells. (A) Study protocol. Eight-week old wild type C57/BL/6 mice were immunized with 50 μg OVA in alum on day 0, followed by intravenous injection of 5×10^5 CD4$^+$ T cells on day 7. Afterwards, the mice received 3% aerosolized OVA on day 8–10 and 10% OVA on day11 for 20 min per day. (B) AHR in response to methacholine was assessed 24 hr after the last exposure to OVA. The result was expressed as means ± SEM (ANOVA, $*p<0.05$). (C) Lungs were stained with hematoxylin and eosin following 0.8 μm cryosection. Analysis was conducted by light microscopy (bar: 100 μm). Data are representative of three independent experiments. (D) Splenocytes taken from the mice were stimulated for 96 hr with 100 μg/ml OVA *in vitro*, after which the cells were washed and restimulated for 4 hr with 50 ng/ml PMA, 1 μM ionomycin and 3 μM Monensin and analyzed by FACS for cytosolic IL-4 and IFN-γ. The data are representative of 3 independent experiments. (E) 48 hr after the exposure with 10% OVA, splenocytes were stimulated for 48 hr with 100 μg/ml OVA , after which cytokine levels in the culture supernatants were determined via ELISA. Data are expressed as the mean ± SEM ($*p<0.05$, *vs.* AIMP1$^{+/+}$ CD4 T group).

(Fig. 4C). Moreover, production levels of IL-4 and IL-5 significantly increased in the mice which received AIMP1-deficient CD4$^+$ T cells, whereas IFN-γ levels remained unchanged (Figs. 4D and E). These results provide direct evidence showing that AIMP1-deficient CD4$^+$ T cells can induce AHR in mice.

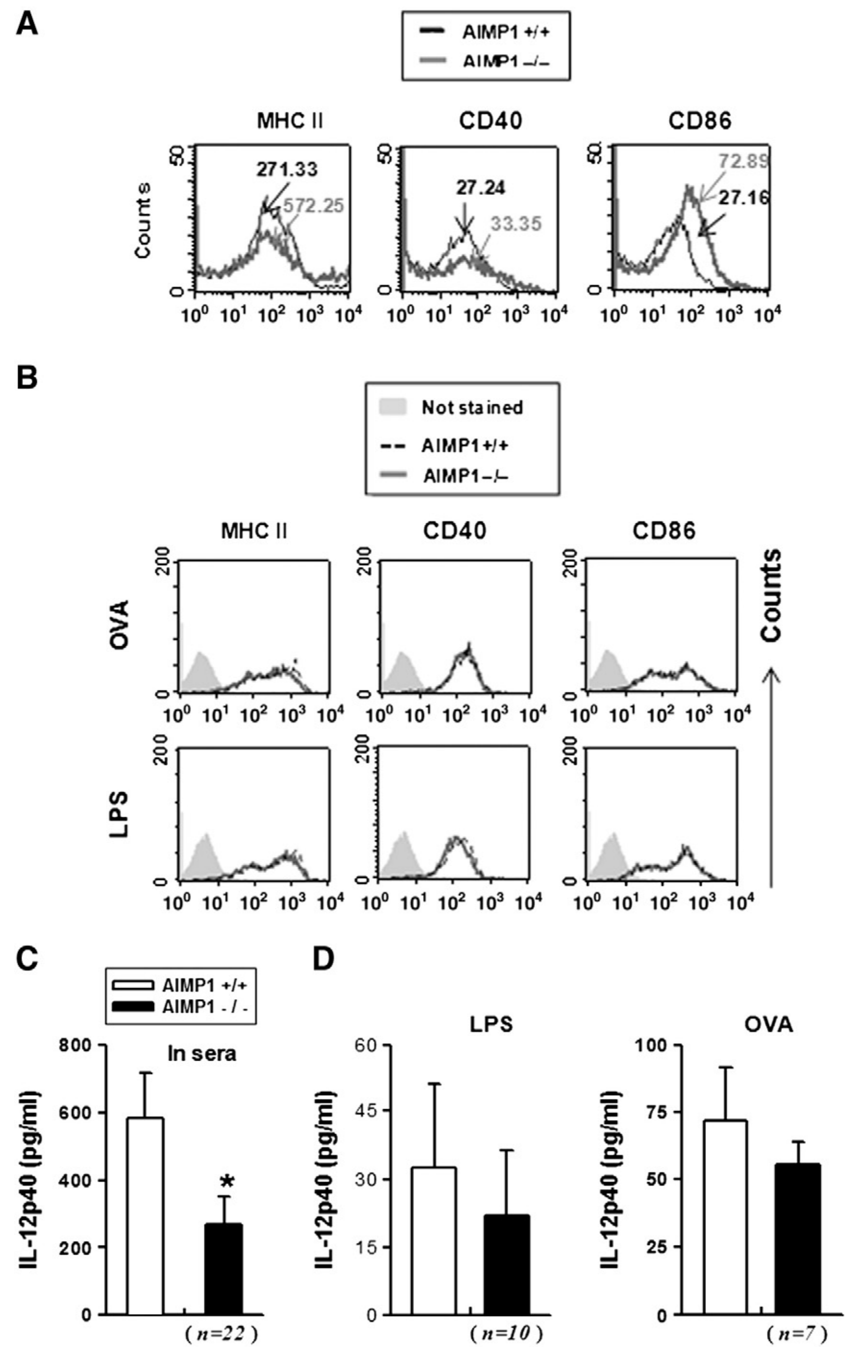

Figure 5 Increased expression of cell-surface molecules and decreased levels of IL-12 production by AIMP1-deficienct DCs. (A) Lung homogenates of 8-week old mice were prepared by enzyme digestion using 1 mg/ml collagenase. Expressions of MHC class II, CD40 and CD86 on CD11c$^+$ cells in homogenates were determined using flow cytometric analysis (black line: wild type, grey line: AIMP1$^{-/-}$). (B) BMDCs were incubated for 18 hr with 50 μg/ml OVA or 500 ng/ml LPS. Expressions of MHC class II, CD40 and CD86 on CD11c$^+$ cells were determined using flow cytometric analysis (shaded area: not stained of wild type BMDCs as a representative of wild type and AIMP1$^{-/-}$ BMDCs, black dotted line: wild type, grey solid line: AIMP1$^{-/-}$). Data in (A) and (B) are expressed as a histogram and are representative of five independent experiments. (C) IL-12p40 levels in sera from wild type and AIMP1-deficient mice were measured by ELISA. Bars show the means ± SEM (*$p<0.05$, *vs* AIMP1$^{+/+}$). (D) IL-12p40 levels in the culture supernatants were determined via ELISA following the stimulation of BMDCs with 500 ng/ml LPS (left) and 50 μg/ml OVA (right) for 18 hr. Data represent the means ± SEM. *$p<0.05$, *vs* AIMP1$^{+/+}$.

3.4. AIMP1-deficiency increases the expression of surface molecules on lung DCs and decreases IL-12p40 production in sera

Previously we reported that AIMP1 protein induces the maturation and activation of BMDCs [17]. Moreover, Han *et al*. reported that ICOSL, CD80 and OX40L were elevated in the absence of AIMP1 [18]. To investigate whether AIMP1-deficiency alters DC functions, we analyzed expression of surface molecules on lung DCs, excluding CD11c$^+$ autofluorescent alveolar macrophages. As shown in Fig. 5A, lung DCs in AIMP1-deficient mice showed dramatically increased expression of MHC class II, CD40 and CD86 molecules compared with those in wild type mice. To investigate whether AIMP1 directly influences the expression of cell surface molecules, immature BMDCs from wild type and AIMP1-deficient mice were incubated with OVA or LPS, and the expression of cell surface molecules were determined via cytofluorometric analysis. As shown in Fig. 5B, AIMP1-deficiency didn't affect the expression of MHC class II, CD40 and CD86 molecules, in contrast to the results observed in lung DCs. Neither the expression of CD80 nor ICOSL was influenced by AIMP1-deficiency (Fig. S2), in contrast to the previous results shown in splenic DCs [18]. Phagocytic ability was not affected by AIMP1-deficiency, either (Fig. S3). These results demonstrate that lung DCs in AIMP1-deficient mice show increased expression of cell surface molecules; however, AIMP1 deficiency didn't affect the maturation and phagocytic function of DCs.

IL-12 is a specific marker of DCs which polarize T cells to a T_H1 phenotype [22]. Interestingly, we observed that IL-12p40 levels in sera were significantly lower in AIMP1-deficient mice than those in wild type mice (Fig. 5C). Furthermore, as shown in Fig. 5D, IL-12p40 production levels were lower in AIMP1-deficient BMDCs upon stimulation with OVA or LPS, compared with that in wild type BMDCs. These data indicate that AIMP1 may be also associated with IL-12p40 production from DCs.

4. Discussion

AIMP1, a novel pleiotropic cytokine, evidences a variety of biological functions by phenotypic investigation related to angiogenesis, wound healing, diabetes and SLE [14,15,23]. In the study, we have demonstrated for the first time that AIMP1 may function as a negative regulator of T_H2-biased responses and airway hyperreactivity.

AIMP1-deficient CD4$^+$ T cells produced significantly higher levels of T_H2 cytokines such as IL-4 and IL-5 than the wild type CD4$^+$ T cells upon OVA- or TCR-stimulation (Fig. 3). Furthermore, increased levels of IFN-γ were produced from the stimulated CD4+ T cells of AIMP1-deficient mice. In contrast, spontaneous airway changes were also reported in T-bet-deficient mice, which showed that T-bet-deficient CD4$^+$ T cells produced increased levels of T_H2 cytokines but decreased levels of IFN-γ [24,25]. The increased IFN-γ production in AIMP1-deficient CD4$^+$ T cells *in vivo* might be due to the compensatory responses against increased T_H2-responses via chronic AIMP1-deficiency and long-lasting spontaneous airway hyperreactivity. In a previous report, the increased levels of IFN-γ and T_H2 cytokines were detected in the chronic phase of asthma patients [26].

GATA-3 expression is critical for the development of T_H2 immune disorder, resulting in IL-4, IL-5 and IL-13 production [27]. In our study the GATA-3 expression in lungs was higher in AIMP1-deficienct mice than in wild type mice, indicating that the increased production of T_H2 cytokines is correlated with the increased GATA-3 expression (Fig. 1B). The interaction between GATA-3 and AIMP1 should be further studied. Furthermore, T_H2 cytokines, in particular IL-5, strongly increased in AIMP1-deficienct mice (Fig. 3), similar to which PARP$^{-/-}$ mice showed reduced IL-5 production specifically [28]. GATA-3 binds to site 3' of *Il4*, the promoters of *Il5*, *Il13* and the *Il4-Il13* intergenic region to produce T_H2 cytokines [29,30]. However, Zhu *et al*. have shown that depletion of GATA-3 completely abolished to generate IL-5 and IL-13 but not IL-4, indicating that each of the T_H2 cytokines can be independently regulated by GATA-3 [31]. However, direct genetic evidence for the differential transcription of T_H2 cytokines remains unclear.

Han *et al*. reported that DCs in AIMP1-deficient mice were characterized as T_H2-inducing cells via increased expression of glycoprotein 96 and ICOSL [18,32,33]. In this study, we showed that expression of MHC class II and costimulatory molecules on lung DCs but BMDCs of AIMP1-deficient mice strongly increased (Fig. 5A). As DCs can be stimulated by DC-activating cytokines such as IL-4, IL-13 and TSLP in a STAT6-dependent pathway [34], this feedback loop may establish the chronicity of allergic inflammation [9]. Based on these previous studies, our data suggest that lung DCs with increased surface markers in AIMP1-deficient mice might be induced by T_H2-dominant milieu which feedback on and stimulate DCs in AIMP1-deficient mice. Therefore, AIMP1-deficient BMDCs which were not present in T_H2-dominant milieu might not show any differences in the expression of cell surface molecules compared to wild type BMDCs. Additionally, AIMP1-deficient mice and BMDCs showed decreased levels of IL-12p40 which is known to abolish AHR and eosinophilia in asthmatic murine model [35]. Reduced IL-12p40 in AIMP1-deficient mice might aid to develop spontaneous allergic airway inflammation. In conclusion, we address a novel function of AIMP1 as a negative regulator of T_H2 immune responses mainly via CD4$^+$ T cells. Additionally, these results strongly indicate that AIMP1 may be an attractive protein as a therapeutic agent for T_H2-biased immune disorder, particularly allergic asthma.

5. Conclusion

Herein, we address the function of AIMP1 as a negative regulator of T_H2 immune responses. These results strongly indicate that AIMP1 may be an attractive protein as a therapeutic agent for T_H2-biased immune disorders, particularly allergic asthma.

Supplementary materials related to this article can be found online at doi:10.1016/j.clim.2012.02.004.

Conflict of Interest Statement

The authors have no financial conflict of interest.

Acknowledgment

We thank to Myun Soo Kim and Dr. Jung Min Han for many helpful insights and animal care. We appreciate G. Trinchieri and S. Wolf for providing valuable reagents. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (2010–0009243).

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