View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Allergology International. 2009;58:445-454
DOI: 10.2332/allergolint.08-OA-0081

ORIGINAL ARTICLE

Effect of BSA Antigen Sensitization during the Acute Phase of Influenza A Viral Infection on CD11c$^+$ Pulmonary Antigen Presenting Cells

Fumitaka Sato[1], Masatoshi Nakazawa[1], Shinichi Yamamiya[1], Chizuru Tamura[1], Naomi Hongo[1], Chie Hotta[1] and Mutsuhiko Minami[1]

ABSTRACT

Background: Influenza A viral infection is concerned with induction of asthma. CD11c$^+$ pulmonary antigen presenting cells (APCs) play a central role in sensitization with inhaled antigens during the acute phase of influenza A viral infection and also reside on bronchial epithelium for the long term after sensitization. To investigate the role of CD11c$^+$ pulmonary APCs in the inhaled antigen sensitization during the acute phase of influenza A viral infection, we analyzed their function.

Methods: Mice were infected with influenza A virus and were sensitized intranasally with BSA/alum during the acute phase of influenza A viral infection. Expression of surface antigens on CD11c$^+$ pulmonary APCs was analyzed by FACS. Cytokine production from CD11c$^+$ pulmonary APCs, and interaction between CD11c$^+$ pulmonary APCs and naïve CD4$^+$ T cells was assessed by ELISA. Ability of antigen presentation by CD11c$^+$ pulmonary APCs was measured by proliferation assay.

Results: BSA antigen sensitization during the acute phase of influenza A viral infection induced eosinophil recruitment into the lungs after BSA antigen challenge and moderately increased expression of MHC class II molecules on CD11c$^+$ pulmonary APCs. The interaction between the CD11c$^+$ pulmonary APCs and naïve CD4$^+$ T cells secreted large amounts of IL-10.

Conclusions: BSA antigen sensitization during the acute phase of influenza A viral infection enhanced IL-10 production from naïve CD4$^+$ T cell interaction with CD11c$^+$ pulmonary APCs. The IL-10 secretion evoked Th2 responses in the lungs with downregulation of Th1 responses and was important for the eosinophil recruitment into the lungs after BSA antigen challenge.

KEY WORDS

airway inflammation, CD4$^+$ T cell, eosinophil, influenza, interleukin-10

INTRODUCTION

Respiratory viral infections are a major factor for the induction and the exacerbation of asthma in infants and in adults.[1-3] Respiratory syncytial virus (RSV) reinfection in neonates is responsible for the development of airway hyperresponsiveness (AHR) and airway eosinophilia via IL-13-dependent pathways.[4] Schwarze *et al*. showed that infection with RSV induces an IL-5-dependent accumulation of eosinophils as a part of the acute inflammatory response in a mouse model.[5] On the other hand, influenza A viral infection leads to the local IFN-γ production by virus-specific T cells with absence of eosinophils for the acute inflammatory response in the lungs.[6-8] Although IFN-γ is known to suppress the differentiation and proliferation of Th2 cells both *in vitro* and *in vivo*,[9,10] several investigators indicated that inhaled antigen sensitization during the acute phase of influenza A viral infection is associated with the induction

[1]Department of Immunology, Yokohama City University School of Medicine, Kanagawa, Japan.
Correspondence: Masatoshi Nakazawa, PhD, Department of Immunology, Yokohama City University School of Medicine, Fukuura 3–9, Kanazawa-ku, Yokohama, Kanagawa 236–0004, Japan.
Email: parasite@med.yokoham–cu.ac.jp
Received 26 December 2008. Accepted for publication 17 March 2009.
©2009 Japanese Society of Allergology

of asthma mediated by Th2 responses.[11-13] In the model of asthma with influenza A viral infection, dendritic cells (DCs) are essential for the induction of Th2 responses.[12,13]

DCs are distributed throughout the lungs, play an important role in antigen presentation, and induce a primary immune response to inhaled antigens. DCs capture inhaled antigens in the airway and migrate to the draining mediastinal lymph nodes, where they move into T cell zones and interact with naïve T cells to initiate antigen-specific immune responses.[14-17] DC maturation occurs following antigen uptake and results in the increased expression of the major histocompatibility complex (MHC) class II and costimulatory molecules, such as CD80/86 and CD40.[18] Also, DCs are associated with the polarization of naïve $CD4^+$ T cells toward Th1 or Th2 cells. The Th1/Th2 cell differentiation of naïve T cells is influenced by the cytokine milieu and the strength of interaction between DCs and naïve T cells.[9,19,20] DCs produce large amounts of IL-12 in response to viral and bacterial stimulation mediated Toll-like receptors. IL-12 secreted by DCs drives polarization toward Th1 cells through binding to IL-12 receptor on naïve T cells.[9,21] Similar to IL-12, IFN-γ plays a central role in the differentiation of Th1 cells.[9] Moreover, high avidity interaction between MHC-peptide complex and T cell receptor (TCR) promotes Th1 cells.[22] In contrast, IL-10 produced by DCs polarizes naïve T cells toward Th2 cells and inhibits the development of Th1 cells.[23] IL-4 production by Th2 cells also enhances the differentiation of naïve T cells toward Th2 cells. Weak TCR-MHC class II interaction between DCs and T cells is thought to induce IL-4-producing T cells.[22]

We have demonstrated that influenza A viral infection enhances the airway sensitization of a suboptimal concentration of antigen. Moreover, influenza A viral infection induced the migration of DCs into the bronchial epithelium, and these migrated cells were essential for the sensitization.[24,25] However, the exact mechanisms of DCs for inhaled antigen sensitization during the acute phase of influenza A viral infection remain still unclear. To investigate how DCs affect on antigen sensitization during the acute phase of influenza A viral infection, we analyzed the function of $CD11c^+$ pulmonary APCs in the asthmatic mouse model.

METHODS

MICE

Female C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan). Ovalbumin $(OVA)_{323-339}$ specific TCR-transgenic (OT-II) mice on a C57BL/6 background were obtained from Jackson Laboratory (Bar Harbor, ME, USA). Animals were fed with OVA free diets and water *ad libitum* and were kept under special pathogen-free conditions in a laminar flow container. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Yokohama City University School of Medicine.

INFLUENZA A VIRAL INFECTION

The mouse-adapted strain of influenza A/PR/8/34 (PR8, H1N1) virus was prepared as previously described (Suzuki *et al.*).[24] Influenza A virus diluted with PBS at a titer of 2×10^3 PFU/ml, which showed virus illumination from day 7 to day 10, was used for infection. Mice were inoculated intranasally with 30 µl of virus solution under anesthesia with diethyl ether.

ALLERGEN SENSITIZATION AND CHALLENGE

Mice were sensitized with aerosolized bovine serum albumin (BSA) (Sigma, St. Louis, MO, USA) solution that was dissolved in PBS at a concentration of 500 µg/ml BSA including 5 mg/ml alum adjuvant (PIERCE, Rockford, IL, USA) for 30 minutes using a DeVilbiss 646 nebulizer (Somerset, PA, USA) during days 3 to 7 after influenza A viral infection. Mice were challenged with aerosolized BSA solution (20 mg/ml BSA in sterilized PBS) for 30 minutes using a DeVilbiss 646 nebulizer during days 28 to 32 after influenza A viral infection.

EXPERIMENTAL GROUPS

The experimental groups were as follows. (I) The control group including mice that were not inoculated with virus on day 0 and were not sensitized with BSA/alum on days 3 to 7. (II) The BSA group including mice that were not inoculated with virus on day 0 and were sensitized with BSA/alum on days 3 to 7. (III) The virus group including mice that were inoculated with virus on day 0 and were not sensitized with BSA/alum on days 3 to 7. (IV) The virus + BSA group including mice that were inoculated with virus on day 0 and were sensitized with BSA/alum on days 3 to 7.

BRONCHOALVEOLAR LAVAGE FLUID (BALF)

Mice were lavaged with 0.8 ml PBS 4 times under anesthesia with pentobarbital sodium (40 mg/kg body weight, Kyoritsu Seiyaku, Tokyo, Japan) on day 34. Cells in the fluid were counted and then were stained with Diff-Quick (International Reagent, Kobe, Japan) for analysis of different leukocyte types. The differential cell count was determined by counting 200 cells under a light microscope.

FLOW CYTOMETRIC ANALYSIS

The following antibodies (Abs) were used in this study; fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD11c mAb (BD Biosciences, San Diego, CA, USA), FITC-conjugated anti-mouse CD86 mAb (eBioscience, San Diego, CA, USA), phycoerythrin (PE)-conjugated anti-mouse MHC class II mAb (Southern Biotechnology Associates Inc., Birmingham, AL, USA), PE-conjugated anti-mouse CD80

mAb (eBioscience), biotinylated anti-mouse CD40 mAb (eBioscience), biotinylated anti-mouse CD11c mAb (eBioscience), biotinylated anti-mouse CD11b mAb (eBioscience), and PE CyCrome 5 (PC5)-conjugated streptavidin (Beckman Coulter, Fullerton, CA, USA). Cells (2×10^6) were stained with Abs described above and were analyzed by FACScan flow cytometer with Cell-Quest Software (BD Biosciences, San Jose, CA, USA).

CELL PREPARATION

To isolate CD11c$^+$ pulmonary APCs, mice were killed at 10 days after influenza A virus infection. Then, lungs were perfused with saline and were minced in RPMI1640 medium (Gibco, Langley, OK, USA) containing 2 mM L-glutamine (Wako, Osaka, Japan), 200 μg/ml streptomycin (Meiji, Tokyo, Japan), and 200 U/ml penicillin (Meiji). These were added with same volume of RPMI1640 medium containing 6% FCS (Biological Industries, Haemek, Israel), 0.4% collagenase (Wako), and 200 μg/ml DNase I (Roche, Mannheim, Germany) for digestion. After digestion, the pieces of lungs were ground with glass slides. Mononuclear cells in the single cell suspension of lungs were collected by Percoll (GE Healthcare Bio-Science Corp., Piscataway, NJ, USA) gradient centrifugation. CD11c$^+$ cells were positively selected by auto-MACS using anti-mouse CD11c MicroBeads (Miltenyi Biotec, Auburn, CA, USA). The purity of CD11c$^+$ cells from lungs was more than 95% according to FACS analysis.

To isolate naïve CD4$^+$ T cells from spleen of OT-II mice (OT-II T cells), spleen was ground with glass slides. The single cell suspension was passed through a nylon fiber column to remove MHC class II$^+$ cells. OT-II T cells were positively selected by auto-MACS using biotinylated anti-mouse CD4 mAb (eBioscience) and anti-biotin MicroBeads (Miltenyi Biotec). The purity of CD4$^+$ cells from spleen of OT-II mice was more than 95% according to FACS analysis.

CYTOKINE PRODUCTION FROM CD11c$^+$ PULMONARY APCs

CD11c$^+$ pulmonary APCs (5×10^5 cells/well) were cultured in 96-well flat-bottom plates in culture medium (RPMI1640 medium supplemented with 10% FCS, 2 mM L-glutamine, 200 μg/ml streptomycin, 200 U/ml penicillin, 10 mM HEPES [Dojindo, Kumamoto, Japan], and 50 μM Mercaptoethanol [Wako]) and were stimulated with 1 μg/ml lipopolysaccharide (LPS) (Sigma) for 48 hours. The culture supernatant was collected and was kept at −80℃ until examined.

PROLIFERATION ASSAY

OT-II T cells (4×10^5 cells/well) were cultured with irradiated CD11c$^+$ pulmonary APCs (1×10^5, 4×10^4, 8×10^3 cells/well) and 100 μg/ml OVA (Sigma) in 96-well flat-bottom plates in the culture medium for 72 hours. To assess the cell proliferation, [^{3}H]-labeled thymidine (0.5 μCi/well) was added for the last 8 hours and the incorporated radioactivity was measured by a Betaplate scintillation counter (Beckman Coulter). All proliferation data represented the average of cpm in triplicate determinations.

CYTOKINE PRODUCTION FROM THE INTERACTION BETWEEN CD11c$^+$ PULMONARY APCs AND OT-II T CELLS

OT-II T cells (4×10^5 cells/well) were cultured with CD11c$^+$ pulmonary APCs (1×10^5 cells/well) and 100 μg/ml OVA in 96-well flat-bottom plates in the culture medium for 72 hours. The culture supernatant was collected and was kept −80℃ until examined.

CYTOKINE ELISAs

Amounts of cytokines in the culture supernatant were determined by IL-4, IFN-γ, and IL-10 ELISA kit according to manufacturer's instructions (eBioscience). Plates were read on a microplate reader (BIO-RAD, Hercules, CA, USA) at a wavelength of 405 nm. The amounts of cytokine were determined by comparing sample values to the linear portion of the standard curve.

STATISTICAL ANALYSIS

Statistical significance of differences between experimental groups was determined by the Student's *t*-test.

RESULTS

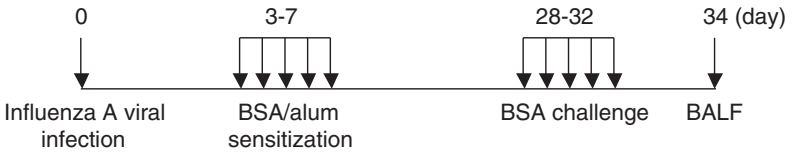
BSA ANTIGEN SENSITIZATION DURING THE ACUTE PHASE OF INFLUENZA A VIRAL INFECTION RECRUITED EOSINOPHILS INTO THE LUNGS AFTER BSA ANTIGEN CHALLENGE

We have shown previously that inhaled antigen sensitization during the acute phase of influenza A viral infection is involved in the induction of asthma.[24,25] We first examined the number of macrophages, lymphocytes, and eosinophils in BALF from each group, which were proceeded using methods indicated in Figure 1a. Mice from the virus + BSA group showed an increased number of macrophages, lymphocytes, and eosinophils into the lungs after BSA antigen challenge. On the other hand, mice from the control group, the BSA group, and the virus group did not enhance the eosinophil recruitment into the lungs after BSA antigen challenge (Table 1).

BSA ANTIGEN SENSITIZATION DURING THE ACUTE PHASE OF INFLUENZA A VIRAL INFECTION ALTERED THE EXPRESSION OF MHC CLASS II MOLECULES ON CD11c$^+$ PULMONARY APCs

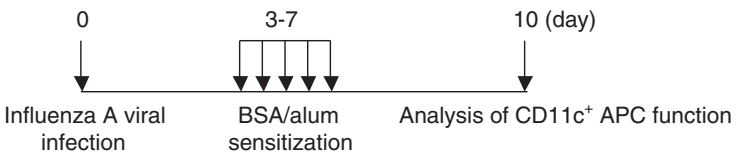
We investigated whether BSA antigen sensitization during the acute phase of influenza A viral infection modify the expression of surface antigens on CD11c$^+$

a



Group	Infection	Sensitization	Challenge
Control	-	-	+
BSA	-	+	+
Virus	+	-	+
Virus + BSA	+	+	+

b



Group	Infection	Sensitization
Control	-	-
Virus	+	-
Virus + BSA	+	+

Fig. 1 Summary of influenza A viral infection, BSA sensitization, and BSA challenge protocols. (**a**) Mice were inoculated with influenza A virus or not inoculated at day 0 and were sensitized with BSA or not sensitized during days 3 to 7. Mice from all groups were challenged with BSA during days 28 to 32 and were lavaged with PBS under anesthesia 48 hours after the last BSA antigen challenge. (**b**) Mice were inoculated with influenza A virus or not inoculated at day 0 and were sensitized with BSA or not sensitized during days 3 to 7. $CD11c^+$ pulmonary APCs from each group were isolated by auto-MACS using anti-mouse CD11c MicroBeads at day 10.

Table 1 The number of macrophages, lymphocytes, and eosinophils in BALF from each group after antigen challenge.

Group	Macrophages ($\times 10^3$)	Lymphocytes ($\times 10^3$)	Eosinophils ($\times 10^3$)
Control	29.1 ± 0.4	0.5 ± 0.4	0.0 ± 0.0
BSA	42.8 ± 3.8	4.4 ± 3.9	0.0 ± 0.0
Virus	43.0 ± 3.0	3.8 ± 3.0	0.0 ± 0.0
Virus + BSA	100.8 ± 22.7*	27.9 ± 22.7*	4.5 ± 4.0*

Mice (n = 5 per group) were inoculated with influenza A virus or not at day 0 and were sensitized with BSA/alum or not during days 3 to 7. Mice from all groups were challenged with BSA during days 28 to 32 and were lavaged with PBS under anesthesia 48h after the last antigen challenge. Cells in BALF were counted and were assessed by Diff-Quick staining. Data are representative of three independent experiments. *$p < 0.05$ compared with the control group or the virus group.

pulmonary APCs isolated at 10 days after influenza A viral infection. Details of each group are shown in Figure 1b. $CD11c^+$ pulmonary APCs from the virus + BSA group showed increased expression of MHC class II, CD40, and CD11b molecules when compared with those of the control group. However, CD80 and CD86 expression on $CD11c^+$ pulmonary APCs showed no difference between either groups. $CD11c^+$ pulmonary APCs from the virus + BSA group and the virus group expressed similar levels of CD80, CD86, CD40, and CD11b. On the other hand, $CD11c^+$ pulmonary APCs from the virus + BSA group showed decreased expression of MHC class II molecules assessed by mean fluorescence intensity (MFI) when compared with those from the virus group (Fig. 2). $CD11c^+$ pulmonary APCs from the BSA group showed similar expression levels of MHC class II, CD40, CD11b, CD80, and CD86 molecules compared with those from the control group (data not shown).

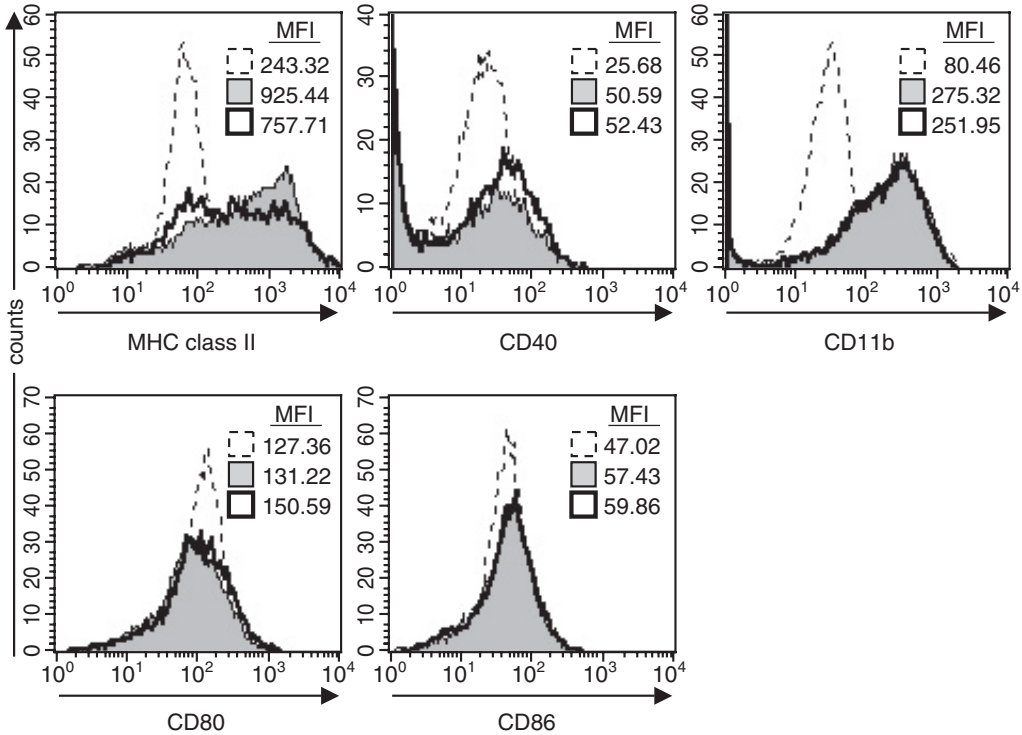


Fig. 2 Influenza A viral infection enhanced the expression of MHC class II, CD40, and CD11b molecules on CD11c+ pulmonary APCs. BSA antigen sensitization during the acute phase of influenza A viral infection showed a moderate expression of MHC class II molecules on CD11c+ pulmonary APCs. CD11c+ pulmonary APCs were positively selected by auto-MACS using anti-mouse CD11c MicroBeads. Cells were stained with PE-conjugated anti-mouse MHC class II mAb, PE-conjugated anti-mouse CD80 mAb, FITC-conjugated anti-mouse CD86 mAb, biotinylated anti-mouse CD40 mAb, biotinylated anti-mouse CD11b mAb, and PC5-conjugated streptavidin and were analyzed by flow cytometry. Three to 10 mice were used in each experimental group. Control group; open histograms with dashed lines, Virus group; shaded histograms with thin lines, Virus + BSA group; open histograms with bold lines. The mean fluorescence intensity is shown in histograms. Data are representative of three independent experiments.

These results indicated that influenza A viral infection increased the expression of MHC class II, CD40, and CD11b molecules on the surface of CD11c+ pulmonary APCs. BSA antigen sensitization during the acute phase of influenza A viral infection decreased the expression levels of MHC class II molecules on CD11c+ pulmonary APCs.

INFLUENZA A VIRAL INFECTION ENHANCED THE ABILITY OF ANTIGEN PRESENTATION BY CD11c+ PULMONARY APCs

We next determined whether the different expression levels of MHC class II molecules on CD11c+ pulmonary APCs from between the virus + BSA group and the virus group affect the ability of antigen presentation. CD11c+ pulmonary APCs were isolated at 10 days after influenza A viral infection. Details of each group were shown in Figure 1b. Naïve CD4+ T cells from OT-II mice were stimulated with CD11c+ pulmonary APCs from each group in the presence of OVA. As expected, CD11c+ pulmonary APCs from the virus + BSA group and the virus group significantly stimulated OT-II T cells compared with those from the control group. CD11c+ pulmonary APCs from the virus + BSA group and the virus group showed similar ability to proliferate OT-II T cells (Fig. 3). The ability of antigen presentation by CD11c+ pulmonary APCs from the BSA group was comparable when compared with those from the control group (data not shown). These results demonstrated that influenza A viral infection enhanced the function of CD11c+ pulmonary APCs in the proliferation of naïve CD4+ T cells. BSA antigen sensitization during the acute phase of influenza A viral infection did not alter the ability of antigen presentation by CD11c+ pulmonary APCs, although the expression levels of MHC class II molecules was different between CD11c+ pulmonary APCs from the virus + BSA group and the virus group.

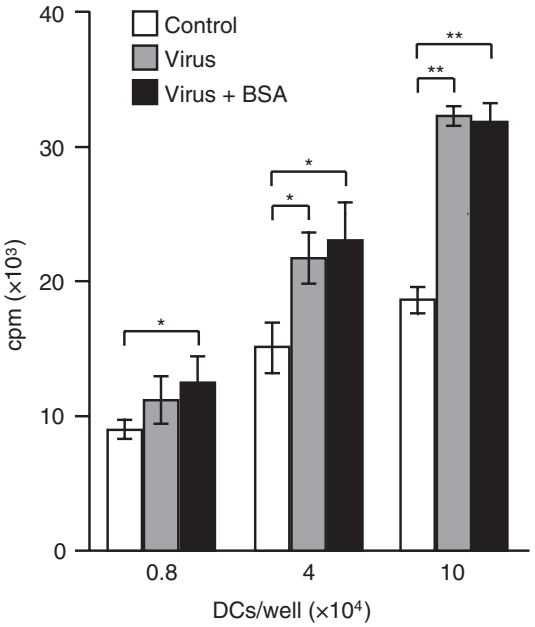


Fig. 3 Influenza A viral infection enhanced ability of antigen presentation of CD11c+ pulmonary APCs. Naïve CD4+ T cells (4×10^5 cells/well) from OT-II mice were cultured with irradiated CD11c+ pulmonary APCs at indicated number from each group in the presence of OVA (100 μg/ml) for 72 hours. Three to 10 mice were used in each experimental group. Control group; white bar, Virus group; gray bar, Virus + BSA group; black bar. Cell proliferation was assessed by incorporation of [^{3}H]-labeled thymidine in T cells. The results are expressed as mean cpm of triplicates ± SD. Data are representative of three independent experiments. *$p < 0.05$, **$p < 0.01$.

BSA ANTIGEN SENSITIZATION DURING THE ACUTE PHASE OF INFLUENZA A VIRAL INFECTION INDUCED INCREASED AMOUNTS OF IL-10 AND IFN-γ PRODUCTION FROM CD11c+ PULMONARY APCs

We next determined if BSA antigen sensitization during the acute phase of influenza A viral infection affected cytokine production from CD11c+ pulmonary APCs isolated at 10 days after influenza A viral infection. Details of each group are shown in Figure 1b. To assess the amounts of their cytokine secretion, they were stimulated with LPS. CD11c+ pulmonary APCs from the virus + BSA group showed significantly larger amounts of IL-10 and IFN-γ secretion when compared with those from the control group. CD11c+ pulmonary APCs from the virus + BSA group produced slightly but not significantly higher amounts of IL-10 compared with those from the virus group.

However, CD11c+ pulmonary APCs from the virus + BSA group produced significantly larger amounts of IFN-γ than those from the virus group. CD11c+ pulmonary APCs from the virus group secreted significantly larger amounts of IL-10 than those from the control group, although IFN-γ production from both CD11c+ pulmonary APCs showed no difference (Fig. 4). CD11c+ pulmonary APCs from the BSA group showed similar ability to secret IL-10 and IFN-γ compared with those from the control group (data not shown). These data suggested that influenza A viral infection enhanced IL-10 production from CD11c+ pulmonary APCs. BSA antigen sensitization during the acute phase of influenza A viral infection led to increased IFN-γ secretion from CD11c+ pulmonary APCs.

BSA ANTIGEN SENSITIZATION DURING THE ACUTE PHASE OF INFLUENZA A VIRAL INFECTION ENHANCED IL-10 PRODUCTION FROM INTERACTION BETWEEN CD11c+ PULMONARY APCs AND NAÏVE CD4+ T CELLS

We next determined whether the interaction between CD11c+ pulmonary APCs from each group and naïve CD4+ T cells from OT-II mice lead to a different cytokine milieu. CD11c+ pulmonary APCs were isolated at 10 days after influenza A viral infection. Details of each group are shown in Figure 1b. OT-II T cells were cultured with CD11c+ pulmonary APCs from each group in the presence of OVA. The amounts of IL-4 in culture supernatant from OT-II T cells cultured with CD11c+ pulmonary APCs from each group were comparable. Interestingly, the interaction between CD11c+ pulmonary APCs from the virus + BSA group and OT-II T cells induced similar ability of IFN-γ production compared with that between CD11c+ pulmonary APCs from the control group and OT-II T cells, and slightly but not significantly less IFN-γ secretion than that between CD11c+ pulmonary APCs from the virus group and OT-II T cells, although CD11c+ pulmonary APCs from the virus + BSA group had significantly larger amounts of IFN-γ production than those from the control group and the virus group as shown in Figure 4 (Fig. 5a). The culture supernatant from OT-II T cells cultured with CD11c+ pulmonary APCs from the virus + BSA group or the virus group showed significantly larger amounts of IL-10 than that from OT-II T cells cultured with CD11 c+ pulmonary APCs from the control group. Moreover, IL-10 production by OT-II T cells cultured with CD11c+ pulmonary APCs from the virus + BSA group was significantly high compared to that by OT-II T cells cultured with CD11c+ pulmonary APCs from the virus group (Fig. 5b). The interaction between CD11c+ pulmonary APCs from the BSA group and OT-II T cells secreted similar amounts of IL-4, IFN-γ, and IL-10 compared with that between CD11c+ pulmonary APCs from the control group and OT-II T cells (data not shown). These results indicated that BSA antigen sensitization during the acute phase of influenza A viral infection enhanced IL-10 production

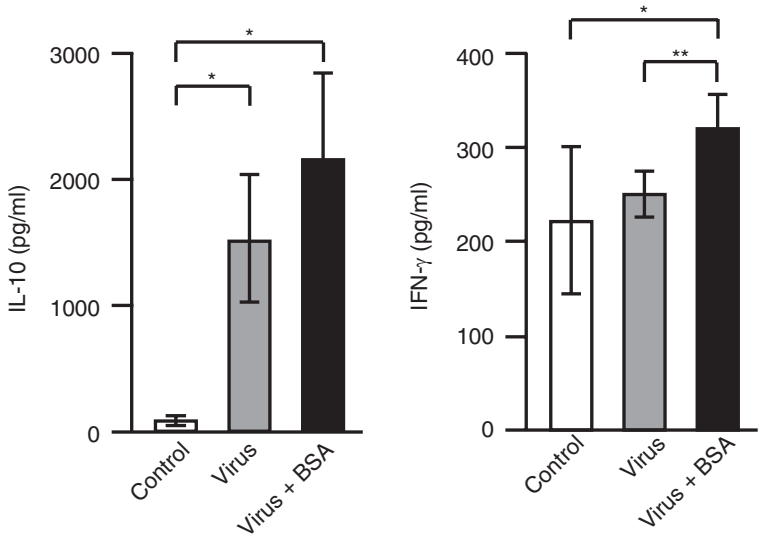


Fig. 4 BSA antigen sensitization during the acute phase of influenza A viral infection induced large amounts of IL-10 and IFN-γ production from CD11c$^+$ pulmonary APCs. CD11c$^+$ pulmonary APCs (5×10^5 cells/well) were cultured with LPS (1 μg/ml) for 48 hours. Three to 10 mice were used in each experimental group. Control group; white bar, Virus group; gray bar, Virus + BSA group; black bar. The culture supernatant was collected and the amounts of IL-10 and IFN-γ were assessed by ELISA kit. *$p < 0.05$, **$p < 0.01$.

by the interaction CD11c$^+$ pulmonary APCs and naïve CD4$^+$ T cells. On the other hand, CD11c$^+$ pulmonary APCs with influenza A viral infection alone differentiated naïve CD4$^+$ T cells into IFN-γ-secreting T cells.

DISCUSSION

In this study, we showed that BSA antigen sensitization during the acute phase of influenza A viral infection recruited eosinophils into the lungs after BSA antigen challenge (Table 1). Influenza A viral infection increased the expression of MHC class II, CD40, and CD11b molecules on the surface of CD11c$^+$ pulmonary APCs and enhanced IL-10 production from CD11c$^+$ pulmonary APCs (Fig. 2 and Fig. 4). Influenza A viral infection also promoted CD11c$^+$ pulmonary APCs to effectively polarize naïve T cells toward IFN-γ-secreting T cells (Fig. 5a). On the other hand, BSA antigen sensitization during the acute phase of influenza A viral infection led CD11c$^+$ pulmonary APCs to express moderate levels of MHC class II molecules (Fig. 2). CD11c$^+$ pulmonary APCs from the virus + BSA group showed the highest ability to secret IL-10 and IFN-γ among those from three groups (Fig. 4). Moreover, the interaction between CD11c$^+$ pulmonary APCs from the virus + BSA group and naïve CD4$^+$ T cells induced more IL-10 production compared with that between CD11c$^+$ pulmonary APCs from the virus group and naïve CD4$^+$ T cells (Fig. 5b). These data demonstrated that BSA antigen sensitization during the acute phase of influenza A viral infection is a key factor for the eosinophil recruitment into the lungs after BSA antigen challenge and may alter Th2-promoting environments in the lungs by downregulation of the Th1 response mediated by IL-10 production from the interaction between CD11c$^+$ pulmonary APCs and naïve CD4$^+$ T cells.

APCs lead to either tolerance or active immune response against antigens by expression of MHC and costimulatory molecules on them.[18] In the lungs, CD11c$^+$ APCs are characterized by decreased expression of MHC and costimulatory molecules, and induction of tolerance at a steady state. Because the respiratory tract is constantly exposed by many innocuous antigens, the peripheral immune system has evolved several mechanisms to maintain a state of tolerance to innocuous antigens in the lungs.[26,27] In this study, CD11c$^+$ pulmonary APCs from the control group showed low expression of MHC class II and CD40 molecules, whereas CD11c$^+$ pulmonary APCs from the virus + BSA group and the virus group exhibited increased expression of MHC class II and CD40 molecules (Fig. 2). Additionally, CD11c$^+$ pulmonary APCs from the virus + BSA group and the virus group showed significantly higher ability to proliferate naïve CD4$^+$ T cells than those from the control group (Fig. 3). These data suggested that CD11c$^+$ pulmonary APCs from the control group are in an immature and induce tolerance to innocuous antigens. In contrast,

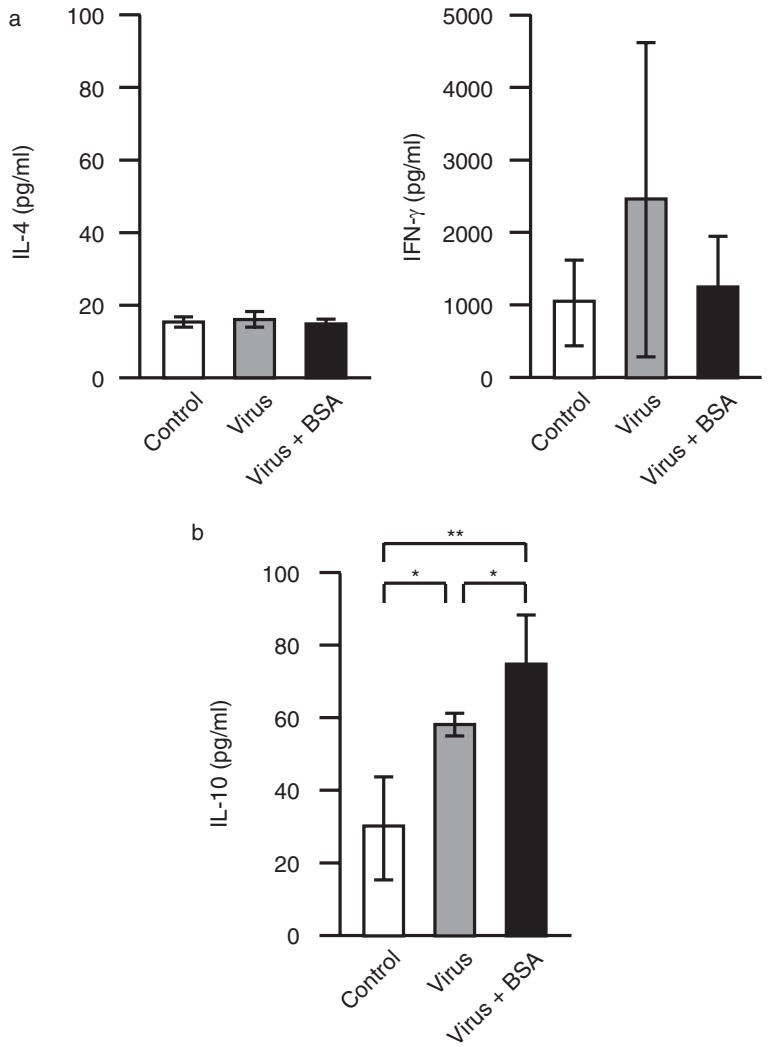


Fig. 5 The interaction between CD11c$^+$ pulmonary APCs from the virus + BSA group and naïve CD4$^+$ T cells showed the largest amounts of IL-10 among those from the three groups. Naïve CD4$^+$ T cells (4×10^5 cells/well) from OT-II mice were cultured with CD11c$^+$ pulmonary APCs (1×10^5 cells/well) in the presence of OVA (100 μg/ml) for 72 hours. Three to 10 mice were used in each experimental group. Control group; white bar, Virus group; gray bar, Virus + BSA group; black bar. The culture supernatant was collected. (**a**) The amounts of IL-4 and IFN-γ were assessed by ELISA kit. (**b**) The amounts of IL-10 were assessed by ELISA kit. Data are representative of three independent experiments. *$p < 0.05$, **$p < 0.01$.

CD11c$^+$ pulmonary APCs from the virus + BSA group and the virus group are in a mature state and lead to active immune response of innocuous antigens. Moderate expression of MHC class II molecules on CD11c$^+$ pulmonary APCs may be caused by large amounts of IL-10 production by BSA antigen sensitization during the acute phase of influenza A viral infection.

Cytokine production from APCs is a key factor for the development or inhibition of immune responses.[9,21] IL-10 has been reported to be a cytokine, which is critical for the suppression of Th1 responses and the induction of Th2 responses.[28,29] CD11c$^+$ pulmonary APCs from the virus + BSA group and the virus group secreted large amounts of IL-10 after LPS stimulation (Fig. 4). Both CD11c$^+$ pulmonary APCs may produce increased amounts of IL-10 secretion to downmodulate the robust Th1 and cytotoxic T lymphocyte responses against influenza A viral infection. Increased amounts of IFN-γ production by CD11c$^+$ pulmonary APCs from the virus + BSA group could play a role in the enhancement of allergic disease *in*

vivo (Fig. 4), although IFN-γ is known to develop Th1 responses.[9] In the mouse model of asthma with influenza A viral infection, injection of neutralizing IFN-γ mAb showed a decreased number of eosinophils into the lungs after BSA antigen challenge and did not enhance the function of pulmonary DCs.[12]

It is well known that IL-10 is a Th2 cytokine as well as an immunosuppressive cytokine, and also inhibits MHC molecules on APCs.[28,29] In this study, BSA antigen sensitization during the acute phase of influenza A viral infection enhanced IL-10 secretion from naïve $CD4^+$ T cells interacted with $CD11c^+$ pulmonary APCs (Fig. 5b). $CD11c^+$ pulmonary APCs from the virus + BSA group had a high ability to proliferate naïve $CD4^+$ T cells (Fig. 3). $CD11c^+$ pulmonary APCs from the virus + BSA group showed moderate expression levels of MHC class II molecules, whereas those from the virus group showed high expression levels of MHC class II molecules (Fig. 2). These data suggested that the IL-10 production from the interaction between $CD11c^+$ pulmonary APCs from the virus + BSA group and naïve $CD4^+$ T cells would enhance Th2 responses and decrease Th1 responses. Additionally, the moderate expression levels of MHC class II molecules on $CD11c^+$ pulmonary APCs from the virus + BSA group could polarize naïve $CD4^+$ T cells toward Th2 cells. A low expression of MHC-peptide complex for TCR leads to Th2 cells, whereas a strong TCR stimulation with increased expression of MHC-peptide complex shows a preference for the induction of Th1 cells.[23] Indeed, BSA antigen sensitization during the acute phase of influenza A viral infection showed an increased number of eosinophils into the lungs after BSA antigen challenge.

In summary, we demonstrated that BSA antigen sensitization during the acute phase of influenza A viral infection induced $CD11c^+$ pulmonary APCs to express moderate levels of MHC class II molecules and large amounts of IL-10 secretion by the interaction between $CD11c^+$ pulmonary APCs and naïve $CD4^+$ T cells. IL-10 produced by the interaction may evoke Th2 responses in the lungs with the downregulation of Th1 responses. Thus, $CD11c^+$ pulmonary APCs from BSA antigen sensitization during the acute phase of influenza A viral infection, at least in part, play an important role in the induction of airway inflammation in our mice model.

REFERENCES

1. Pattemore PK, Johnston SL, Bardin PG. Viruses as precipitants of asthma symptoms. I. Epidemiology. *Clin Exp Allergy* 1992;**22**:325-36.
2. Frick OL, German DF, Mills J. Development of allergy in children. I. Association with virus infections. *J Allergy Clin Immunol* 1979;**63**:228-41.
3. Beasley R, Coleman ED, Hermon Y, Holst PE, O'Donnell TV, Tobias M. Viral respiratory tract infection and exacerbations of asthma in adult patients. *Thorax* 1988;**43**:679-83.
4. Dakhama A, Park JW, Taube C *et al*. The enhancement or prevention of airway hyperresponsiveness during reinfection with respiratory syncytial virus is critically dependent on the age at first infection and IL-13 production. *J Immunol* 2005;**175**:1876-83.
5. Schwarze J, Hamelmann E, Bradley KL, Takeda K, Gelfand EW. Respiratory syncytial virus infection results in airway hyperresponsiveness and enhanced airway sensitization to allergen. *J Clin Invest* 1997;**100**:226-33.
6. Doherty PC, Topham DJ, Tripp RA, Cardin RD, Brooks JW, Stevenson PG. Effector CD4+ and CD8+ T-cell mechanisms in the control of respiratory virus infections. *Immunol Rev* 1997;**159**:105-17.
7. Roman E, Miller E, Harmsen A *et al*. CD4 effector T cell subsets in the response to influenza: heterogeneity, migration, and function. *J Exp Med* 2002;**196**:957-68.
8. Doyle AG, Buttigieg K, Groves P, Johnson BJ, Kelso A. The activated type 1-polarized CD8(+) T cell population isolated from an effector site contains cells with flexible cytokine profiles. *J Exp Med* 1999;**190**:1081-92.
9. O'Garra A. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* 1998;**8**:275-83.
10. Gajewski TF, Fitch FW. Anti-proliferative effect of IFN-gamma in immune regulation. I. IFN-gamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. *J Immunol* 1988;**140**:4245-52.
11. Yamamoto N, Suzuki S, Suzuki Y *et al*. Immune response induced by airway sensitization after influenza A virus infection depends on timing of antigen exposure in mice. *J Virol* 2001;**75**:499-505.
12. Dahl ME, Dabbagh K, Liggitt D, Kim S, Lewis DB. Viral-induced T helper type 1 responses enhance allergic disease by effects on lung dendritic cells. *Nat Immunol* 2004;**5**:337-43.
13. Marsland BJ, Scanga CB, Kopf M, Le Gros G. Allergic airway inflammation is exacerbated during acute influenza infection and correlates with increased allergen presentation and recruitment of allergen-specific T-helper type 2 cells. *Clin Exp Allergy* 2004;**34**:1299-306.
14. Austyn JM. New insights into the mobilization and phagocytic activity of dendritic cells. *J Exp Med* 1996;**183**:1287-92.
15. Larsen CP, Steinman RM, Witmer-Pack M, Hankins DF, Morris PJ, Austyn JM. Migration and maturation of Langerhans cells in skin transplants and explants. *J Exp Med* 1990;**172**:1483-93.
16. Xia W, Pinto CE, Kradin RL. The antigen-presenting activities of Ia+ dendritic cells shift dynamically from lung to lymph node after an airway challenge with soluble antigen. *J Exp Med* 1995;**181**:1275-83.
17. Steinman RM, Pack M, Inaba K. Dendritic cells in the T-cell areas of lymphoid organs. *Immunol Rev* 1997;**156**:25-37.
18. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998;**392**:245-52.
19. Seder RA, Paul WE. Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Annu Rev Immunol* 1994;**12**:635-73.
20. Constant SL, Bottomly K. Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. *Annu Rev Immunol* 1997;**15**:297-322.
21. Macatonia SE, Hosken NA, Litton M *et al*. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+ T cells. *J Immunol* 1995;**154**:5071-9.
22. Badou A, Savignac M, Moreau M *et al*. Weak TCR stimu-

lation induces a calcium signal that triggers IL-4 synthesis, stronger TCR stimulation induces MAP kinases that control IFN-gamma production. *Eur J Immunol* 2001;**31**: 2487-96.

23. Iwasaki A, Kelsall BL. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J Exp Med* 1999;**190**:229-39.

24. Suzuki S, Suzuki Y, Yamamoto N *et al*. Influenza A virus infection increases IgE production and airway responsiveness in aerosolized antigen-exposed mice. *J Allergy Clin Immunol* 1998;**102**:732-40.

25. Yamamoto N, Suzuki S, Shirai A *et al*. Dendritic cells are associated with augmentation of antigen sensitization by influenza A virus infection in mice. *Eur J Immunol* 2000; **30**:316-26.

26. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol* 2001;**2**:725-31.

27. Holt PG. Antigen presentation in the lung. *Am J Respir Crit Care Med* 2000;**162**:S151-6.

28. Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J Exp Med* 1989;**170**:2081-95.

29. Laouini D, Alenius H, Bryce P *et al*. IL-10 is critical for Th2 responses in a murine model of allergic dermatitis. *J Clin Invest* 2003;**112**:1058-66.