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Role of Bombesin Receptor Activated Protein in the Antigen Presentation by Human Bronchial Epithelial Cells

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ABSTRACT

Bombesin receptor activated protein (BRAP) was identified in a bacterial two-hybrid screen for proteins interacting with bombesin receptor subtype-3 (BRS-3). We found that BRAP is widely expressed in the airway epithelium of human lungs and may play a role during the stress response of lung epithelium. In this work, we explored the potential roles of BRAP in the antigen presenting function of human bronchial epithelial cells (HBECs). Overexpression of a BRAP recombinant protein in a human bronchial epithelial cell line resulted in a reduction of FITC-OVA uptake by HBECs, which indicated that the antigen uptake ability is inhibited. The analysis of the protein expression of surface molecules including B7 homologs and the major histocompactability complex (MHC) class II molecules showed that the expression levels of HLA-DR and B7DC increased while the levels of B7-H1 and B7.2 decreased. Since those surface molecules are all related to antigen presenting process, the altered expression pattern of those molecules provides further evidence showing that BRAP overexpression leads to a change in antigen presenting function of HBECs. Moreover, overexpression of BRAP in HBECs caused a decrease of co-cultured lymphocytes proliferation and a changed pattern of cytokines produced by lymphocytes in the presence of FITC-OVA, which indicated that changes in the maturation pattern and functions of co-cultured lymphocytes were induced by BRAP overexpression. Overall, our results suggested that overexpression of BRAP may play a role during the antigen presenting process of bronchial epithelium by inhibiting the antigen uptake ability of bronchial epithelial cells. J. Cell. Biochem. 114: 238–244, 2013.

KEY WORDS: BRS-3; BRAP; ANTIGEN PRESENTATION; HUMAN BRONCHIAL EPITHELIAL CELLS

Bronchial epithelial cells line the surface of airways, forming the first barrier to the external environment and play a critical role in the stabilization of airway microenvironment homeostasis. Increasing evidence indicates that bronchial epithelial cell lining is not merely a simple mechanic barrier that segregates the outside environment from the internal environment, but is also engaged in the regulation of local immune responses by producing cytokine and chemokines [Pichavant et al., 2003; Liu et al., 2010]. Moreover, bronchial epithelial cells express major histocompatibility complex (MHC) class I and class II molecules and co-stimulatory molecules such as the B7 family and toll like receptors. Therefore, they may also function as non-specialized antigen-presenting cells (APC), presenting antigen to T lymphocytes and stimulating the proliferation and differentiation of T lymphocytes [Salik et al., 1999; Papi et al., 2000].

Bombesin receptor subtype-3 (BRS-3) is an orphan bombesin receptor, which may play an important role in the regulation of stress responsiveness in lung and airway epithelium [Tan et al., 2006, 2007]. Bombesin receptor activated protein (BRAP) is a novel protein that can interact with BRS-3 as identified by a bacterial two-

Xiangping Qu and Menglan Li contributed equally to this work.

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hybrid screen [Liu et al., 2011]. In that study of an airway hyperresponsiveness (AHR) animal model, we found that both mRNA and protein expression levels of BRAP increased significantly in lung tissue induced by ozone stress for 2–4 days suggesting an important role during the stress response to airway inflammation and AHR. In the same study, we found by confocal laser scanning microscopy that BRAP protein localized to membrane and cytoplasm. BRAP over-expression resulted in increased S and G2 phase of cell cycle, promoted the ability of cell repair, and enhanced proliferation of human bronchial epithelial cells (HBECs) [Liu et al., 2011].

Our group also found that BRS-3 may have impact on the antigen presenting process of HBECs by enhancing the antigen uptaking ability of those cells (data not published). Since BRAP is a protein that can interact with BRS-3, we further investigated the potential roles of BRAP in the antigen presenting function of HBECs by introducing a pcDNA3.1(+)/BRAP mammalian recombinant expression plasmid [Liu et al., 2011] into a HBEC cell line using stable transfection techniques. Subsequent studies of BRAP in HBECs addressed its role on antigen uptake and antigen presentation. Our results show that overexpression of BRAP in HBEC inhibit antigen uptake and change the expression pattern of surface molecules related to antigen presenting process. In addition, we demonstrate that BRAP over-expression in HBECs inhibits the proliferation of co-cultured human lymphocytes and lymphocytes-dependent secretion of cytokines such as IL-4 and IL-17.

MATERIALS AND METHODS

CELL CULTURE AND STABLE TRANSFECTION

The immortalized human bronchial epithelial cell line 16HBE14o- (a kind gift of Dr. Gruenert, University of California, San Francisco) was maintained in a mixture media of DMEM:F12 (1:1) supplemented with 10% heat-inactivated newborn bovine serum, 100 μg/ml penicillin, and 100 μg/ml streptomycin. Cells were incubated at 37°C in a humidified 5% CO_2 atmosphere and then referred to as HBECs for the following experiments.

The human BRAP protein was expressed from plasmid pcDNA3.1(+)/BRAP as described in detail previously [Liu et al., 2011]. The plasmid pcDNA3.1(+)/BRAP and the control vector plasmid pcDNA3.1(+) were transfected into 16HBE14o- cells using FuGENE® HD Transfection Reagent according to the manufacturer's protocol (Roche Applied Science, 68298, Mannheim, Germany). Transfected cells were grown for 36 h in medium containing 600 μg/ml G418 followed by selection of resistant clones. After 4 weeks, positive clones were selected, isolated, and maintained in complete culture media containing 200 μg/ml G418.

TOTAL RNA ISOLATION AND REAL-TIME PCR ANALYSIS

Real-time PCR was performed to determine BRAP mRNA expression after transfection and mRNA expression of surface molecules including B7 costimulatory molecules and HLA-DR after BRAP was overexpressed in 16HBE14o- cells. Total RNA was extracted from pcDNA3.1(+)/BRAP stably transfected cells, pcDNA3.1(+) control vector stably transfected cells and cells without transfection, respectively, using TRIzol reagent (Invitrogen, Carlsbad, CA). One mirogram total RNA was subjected to reverse transcription into first strand cDNA using Revert Aid™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Rockford, IL). The sequences of PCR primers used for BRAP were: forward, 5′-GCTGCTGGAAAAGAATGAACC-3′; reverse, 5′-TGGCAATGGGCAAGGACA-3′; primers for GAPDH were: forward, 5′-CCACTCCTCCACCTTTGAC; reverse, 5′-ACCCTGTTGCTGTAGCCA-3′. The sequences of the primers used for amplifying B7 costimulatory molecules and HLA-DR are shown in Table I. The real-time PCR analyses were performed with a SYBR Green I Assay kit (Bio-Rad Applied Biosystems), and the PCR amplification reaction was performed as follows: 94°C for 4 min, followed by 40 cycles of 94°C for 30 s and 60°C for 30 s. The negative control was performed as an equal volume of water substituted for the reverse transcriptase in the PCR reaction, and the normalization of mRNA expression was achieved by comparing the copy numbers of mRNA of each detected molecule with that of humanβ-actin.

PROTEIN EXTRACTION AND WESTERN BLOTTING

BRAP protein expression levels after transfection were evaluated by Western blotting analysis. Total protein was extracted from the pcDNA3.1(+)/BRAP stably transfected cells, pcDNA3.1(+) control vector stably transfected cells and cells without transfection, respectively, and the protein concentrations were measured by BCA protein quantification kit. The partial polyclonal antibody against human BRAP was obtained from GenScript, Nanjing, China. The antigen sequence within BRAP to develop this antibody is GGDEDRPFPDFDPW. After heating at 95°C for 10 min, equal amounts of total protein was separated by 7.5% SDS–PAGE and subsequently transferred onto PVDF membrane. The membrane was washed, blocked with 2% BSA–TBS for 2 h, and then incubated with primary rabbit anti-human BRAP polyclonal antibody (1:200 dilution) at 4°C overnight. After washing, the membranes were incubated with a secondary horseradish peroxidase-labeled goat anti-rabbit antibody (1:1,000) at 37°C for 2 h, and then washed with TTBS buffer for three times. BRAP specific expression was visualized with enhanced chemiluminescene system (Thermo Fisher Scientific). β-Actin expression of the equal amount of above samples was also measured by Western blot using anti-β-actin antibody (BD Biosciences, San Jose, CA). Then the protein expression level was

TABLE I. Primers Used for Real-Time PCR Analysis

Primers	Sequences of primers
B7-1-human	Forward: 5′-GGAGGCAGGGAACATCACC-3′ Reverse: 5′-CCCCAGACATCATAGTCAGCA-3′
B7-2-human	Forward: 5′-GTTTTGATTCGGACAGTTGGAC-3′ Reverse: 5′-TGGGTAACCGTGTATAGATGAGC-3′
ICOSL-human	Forward: 5′-ACCTACCACATCCCACAGAACA-3′ Reverse: 5′-CCTCAACGCTCAAAACCTCC-3′
PD-L1-human	Forward: 5′-CCGAAGTCATCTGGACAAGCA-3′ Reverse: 5′-AAGGCATAATAAGATGGCTCCC-3′
PDL2-human	Forward: 5′-TGCATAATCATCTATGGGGTCG-3′ Reverse: 5′-CTGGAGTGGCTGGTGTTGG-3′
B7-H3-human	Forward: 5′-ACAGGGCAGCCTATGACATTC-3′ Reverse: 5′-CAGCTCCTGCATTCTCCTCC-3′
B7-H4-human	Forward 5′- GAGGCTCCCCGATGGTTC-3′ Reverse 5′- CCTTTTGATCTCCGATTCTGTC-3′
HLA-DR-human	Forward: 5′-AAAGAAGGAGACGGTCTGGC-3′ Reverse: 5′-TGGGGTGAACTTGTCTATGAAAC-3′
β-Actin-human	Forward: 5′-TGACGTGGACATCCGCAAAG-3′ Reverse: 5′-CTGGAAGGTGGACAGCGAGG-3′

quantified by measuring protein band density with Bio-Rad Gel Doc™ XR system and the expression level of BRAP was normalized by comparing BRAP expression with β-actin expression.

ASSESSMENT OF THE ANTIGEN UPTAKE ABILITY

pcDNA3.1(+)/BRAP recombinant plasmid, pcDNA3.1(+) control vector plasmid stably transfected HBECs and normal HBECs were seeded in 24- or 6-well plates and cultured to 80% confluence. Cells were incubated with FITC-labeled ovalbumin (FITC-OVA, 100 μg/ml, Molecular Probes) at 37 °C and 5% CO_2 for 30, 60, and 90 min, respectively. Finally, cells of different groups were harvested and washed at different time points, and observed under a fluorescence microscope or evaluated by flow cytometry. The mean fluorescence intensity (MFI) or percentage of positive cells (internalized FITC-OVA) were obtained and they were used as indicators of the antigen uptaking ability of HBECs.

FLUORESCENCE ACTIVATED CELL SORTER (FACS) ANALYSIS FOR B7 COSTIMULATORY MOLECULES AND HLA-DR EXPRESSION

pcDNA3.1(+)/BRAP, or pcDNA3.1(+) control vector plasmid stably transfected cells and cells without transfection were seeded in 6-well plates and cultured to 80% confluence. 1×10^6 cells of each sample were harvested and washed twice with PBS. After removal of the supernatant, cells were resuspended in 100 μl PBS containing 20 μl PE-labeled anti-human B7.2, B7-H1, B7-DC, and HLA-DR monoclonal antibodies (BD Biosciences), respectively. After 30 min incubation at 4°C, the excess antibodies were removed by PBS washing and centrifugation at 1,500 rpm for 5 min. Cells were then resuspended in 500 μl PBS and analyzed immediately by flow cytometry using CellQuest software. Data represent the measurement of mean fluorescence intensity (MFI) or percentage of at least 10,000 positive cells and was compared with the control groups.

ISOLATION OF LYMPHOCYTES

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors by density gradient centrifugation using Ficoll-Hypaque lymphocyte separation media. The isolated PBMCs were washed twice with PBS and resuspended in RPMI 1640 medium containing 10% FCS, then incubated in the culture flasks at 37°C for 2 h. After that, the monocytes adhere to the culture flasks and the nonadherent lymphocyte cells were collected, washed, and resuspended in RPMI 1640 complete medium for further experiment.

CO-CULTURE OF LYMPHOCYTES AND 16HBE14O- CELLS

Airway epithelial cells have the capacity to present antigens to and stimulate T cells in vitro, and the epithelial cells can be co-cultured with T cells since T cells can proliferate in the supernatants in the wells containing HBECs [Oei et al., 2004; Liu et al., 2012] 16HBE14o- cells of different groups were seeded in 6-well plates at 1×10^5 cells per well. When cells reached 50% confluence, they were pretreated with 1 mg/ml ovalbumin (OVA, Sigma) for 2 h and washed twice with PBS to remove the redundant OVA. Then 1×10^6 freshly isolated lymphocytes were added to each well. After co-culture with HBECs for 48 h, the lymphocytes were subjected to cell cycle analyses, and IFN-γ, IL-4, and IL-17 levels measured in supernatants by ELISA technique.

CELL CYCLE ANALYSIS OF LYMPHOCYTES

After co-culture with 16HBE14o- cells for 48 h, lymphocytes of different groups were harvested and washed twice, and then fixed with pre-cooled 70% ethanol at 4°C overnight. On the next day, the lymphocytes were washed twice with PBS, digested with 50 μg/ml DNase-free RNAse, and stained with 50 μg/ml propidium iodide solution at 4°C for 45 min. The cell cycle of lymphocytes was then immediately analyzed by flow cytometry and the populations of G1, S, G2, and G2/G1 cells were quantified.

THE MEASUREMENT OF CYTOKINES

The levels of the cytokines IFN-γ, IL-4, and IL-17 in the supernatants of the co-cultured cell system were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instruction (R&D Systems).

STATISTICAL ANALYSIS

All described experiments were repeated three times. Data are presented as means ± SEM. Comparisons between three experimental groups were performed by one-way analysis of variance (ANOVA), followed by SNK test using SPSS 13.0 software. For comparison of two groups, the Student's *t*-test was used, and a value of $P < 0.05$ was considered as statistically significant.

RESULTS

BRAP IS OVEREXPRESSED IN STABLY TRANSFECTED HBECs

To explore the influence of BRAP on the antigen presenting ability of bronchial epithelial cells, we established an epithelial cell model of BRAP overexpression by stably transfecting an immortalized human bronchial epithelial cell line 16HBE14o- with plasmid pcDNA3.1(+)/BRAP. BRAP is constitutively expressed in human lungs and 16HBE14o- cells as shown in our previous study [Liu et al., 2011]. Results of real-time PCR (Fig. 1A) showed that the expression of BRAP mRNA in the pcDNA3.1(+)/BRAP stably transfected cells increased significantly compared with control cells stably trasfected with pcDNA3.1(+) control vector plasmid.

The BRAP protein levels of different groups were examined by Western blot analysis and normalized with levels of human β-actin of the same sample (Fig. 1B). Stable transfection of plasmid pcDNA3.1(+)/BRAP resulted in much more abundant BRAP protein expression compared with cells transfected with control vector and the difference of BRAP protein levels between cells transfected with pcDNA3.1(+)/BRAP and control vector is significant ($P < 0.05$).

BRAP OVEREXPRESSION INHIBITS THE ANTIGEN UPTAKE ABILITY OF HBECs

To investigate the ability of HBECs of antigen uptake, we analyzed 16HBE14o- cells of different groups with fluorescence microscopy subsequent to incubation with FITC-OVA for various periods. Specific, green fluorescence signals appeared within cells after incubation for 30, 60, and 90 min as shown in Figure 2A. To analyze the antigen uptake ability of HBECs, we compared the MFI values of

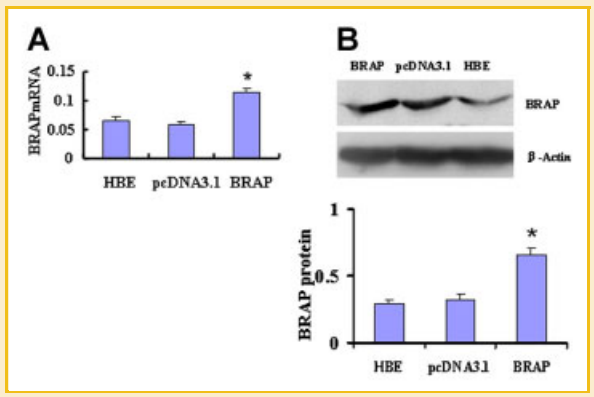

Fig. 1. BRAP over-expression in HBECs. Cells were stably transfected with vector alone and pcDNA3.1(+)/BRAP expression plasmid. Then BRAP mRNA and protein expression was determined. Panel A (left) shows BRAP mRNA expression as determined by real-time PCR in normal HBECs (depicted as HBE), pcDNA3.1(+) control vector transfected HBECs (depicted as pcDNA3.1), and pcDNA3.1(+)/BRAP transfected HBECs (depicted as BRAP). The results represent the mean of 3 independent experiments and are normalized relative to β-actin mRNA expression ($^{*}P < 0.05$ vs. pcDNA3.1(+)). Panel B (right) depicts BRAP protein expression and β-actin loading controls as determined by Western blot analysis. The results represent the mean of 3 independent experiments and are normalized relative to β-actin expression. HBEC with BRAP overexpressed (BRAP) have increased BRAP levels when compared with control vector transfected HBECs ($^{*}P < 0.05$ vs. pcDNA3.1(+) vector control).

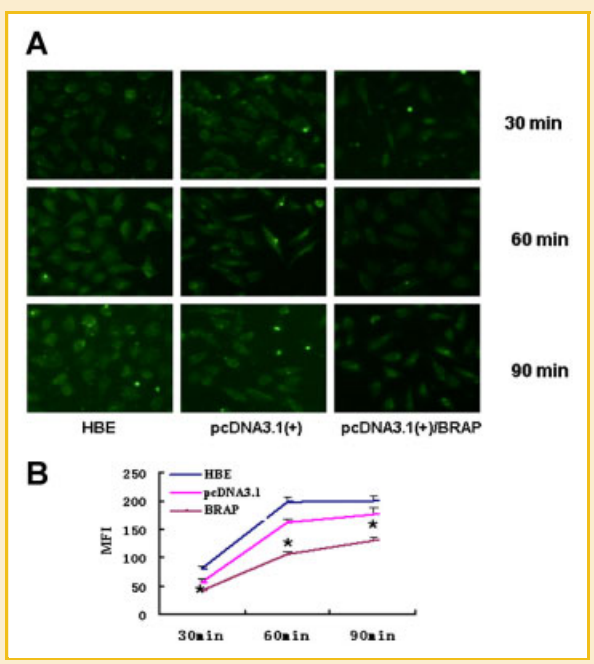

Fig. 2. BRAP overexpression reduces FITC-OVA antigen uptake in HBECs. Panel A (top) depicts endocytosed, fluorescently labeled FITC-OVA in normal HBECs (HBE), pcDNA3.1(+) control transfected HBECs, and pcDNA3.1(+)/BRAP transfected HBECs (BRAP) after incubation for 30, 60, and 90 min. Panel B (bottom) shows the MIF values of fluorescently labeled, endocytosed FITC-OVA as detected by flow cytometry in normal HBECs (HBE), pcDNA3.1(+) control HBECs (pcDNA3.1(+)), and pcDNA3.1(+)/BRAP HBECs (BRAP) incubation for 30, 60, and 90 min ($^{*}P < 0.05$ vs. pcDNA3.1(+) vector control).

the endocytosed FITC-OVA in 16HBE14o- cells of different groups. In contrast to cells transfected with control vector, BRAP overexpressed cells showed a significant decrease in MFI at different time points, suggesting that BRAP might inhibit the antigen uptake ability of bronchial epithelial cells (Fig. 2B).

BRAP OVEREXPRESSION RESULTS IN ALTERED EXPRESSION PATTERN OF B7 FAMILY AND HLA-DR ON THE SURFACE OF 16HBE14O- CELLS

Antigen presenting cells are characterized in part by the presence of specific cell surface molecules that aid in antigen presentation on the cell surface and allow interactions with the T-cell receptor complex [Collins et al., 2005; Greenwald et al., 2005]. The important surface molecules includes co-stimulatory molecules B7 family and MHC-II molecules. To determine the effect of BRAP in HBECs on the expression of cell surface molecules related to antigen presenting process, the mRNA levels of B7 family molecules and HLA-DR, an important MHC II molecule, were examined by real-time PCR first. As shown in Figure 3, BRAP overexpression in HBECs resulted in significant up-regulation of mRNA levels of B7.1, B7.2, B7-H1, B7-H2, B7-H3, B7-H4, B7-DC, and HLA-DR.

Since our another study showed that 16HBE14o- cells only expressed B7.2, B7-H1, B7DC, and HLA-DR molecules (not published), we then determined the protein expression of B7.2, B7-H1, B7DC, and HLA-DR on the surface of 16HBE14o- cells by flow cytometry analysis. However, the protein expression pattern of those surface molecules related to antigen presenting process is different from mRNA expression pattern. BRAP overexpression yielded significantly increased HLA-DR and B7DC surface protein expression, but decreased B7-H1 and B7.2 (CD86) expression ($P < 0.05$, as shown in Fig. 4).

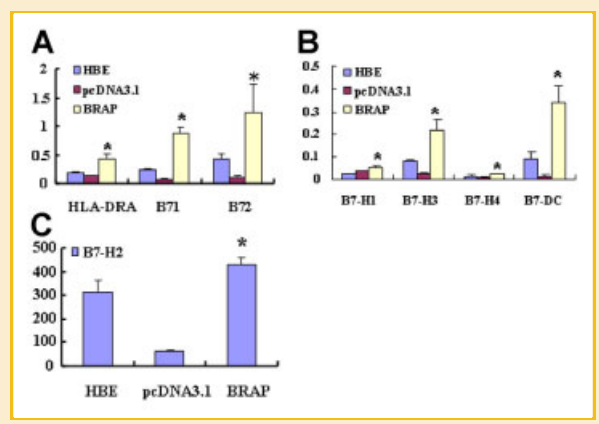

Fig. 3. BRAP affects m RNA expression of HLA-DR and B7 co-stimulatory molecules in HBECs. The mRNA levels of HLA-DR, B71, and B72 (Top left panel, A); B7-H1, B7-H3, B7-H4, and B7DC (Top right panel, B); and B7-H2 (Bottom panel, C) were determined by real-time PCR in normal HBECs (HBE), pcDNA3.1(+) controltransfected HBECs, and pcDNA3.1(+)/BRAP transfected HBECs (BRAP). Results represent means ± SEM of 3 experiments and are normalized to GAPDH mRNA expression; $^{*}P < 0.05$ versus pcDNA3.1(+) vector control.

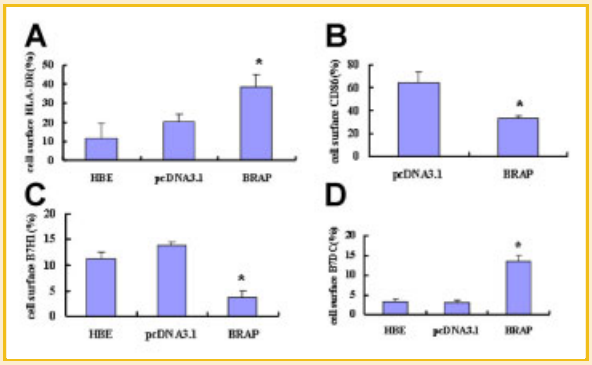

Fig. 4. Determination of HLA-DR and B7 co-stimulatory molecule expression on HBECs by flow cytometry. The expression of HLA-DR (A), CD86 (B), B7H1 (C), and B7DC (D) in normal HBECs (HBE), pcDNA3.1(+) control HBECs (pcDNA3.1), and pcDNA3.1(+)/BRAP transfected HBECs (BRAP) was detected by flow cytometry. Results are shown as mean + SEM of 3 experiments. BRAP induces the expression of HLA-DR and B7DC, while it inhibits the expression of CD86 and B7H1, $^{*}P < 0.05$ versus pcDNA3.1(+) vector control.

BRAP OVEREXPRESSION REGULATES CELL CYCLE IN CO-CULTURED LYMPHOCYTES

As compared with lymphocytes co-cultured with HBECs transfected with vector control plasmid and untransfected HEBCs, lymphocytes co-cultured with HBECs overexpressing BRAP exhibited a decreased percentage in S phase (Fig. 5A) and an increase in G1 phase (Fig. 5B). These results indicate that BRAP overexpressed in HBECs may cause a change in the maturation and proliferation pattern of co-cultured lymphocytes.

BRAP OVEREXPRESSION IN HBECs INHIBITS IL-4 AND IL-17 SECRETION FROM CO-CULTURED LYMPHOCYTES

Since BRAP overexpression in HBECs caused cell cycle changes in the co-cultured lymphocytes and the lymphocytes isolated were composed mainly of T cells, we postulated that the BRAP overexpression may also cause a change in the T cell functions by altering antigen presenting ability of HBECs. To explore this

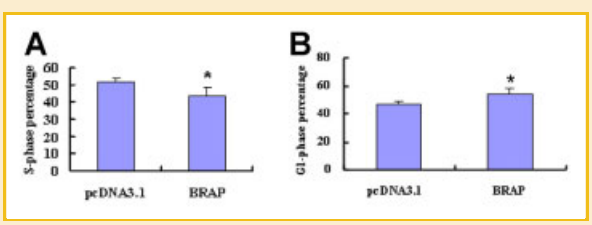

Fig. 5. BRAP regulates cell cycle phases in lymphocytes co-cultured with HBECs. Cell cycle phases of freshly isolated lymphocytes were analyzed by flow cytometry after co-culture with BRAP-over-expressing HBECs. pcDNA3.1(+) vector control (pcDNA3.1) and pcDNA3.1(+)/BRAP transfected HBECs (BRAP) were challenged with OVA for 2 h and then co-cultured with freshly isolated lymphocytes for 48 h. Lymphocytes were then harvested, stained with PI, and immediately analyzed by flow cytometry. The cell cycle population in S phase (A) and G1 phase (B) were quantified from the DNA histograms ($^{*}P < 0.05$ vs. pcDNA3.1(+) vector control).

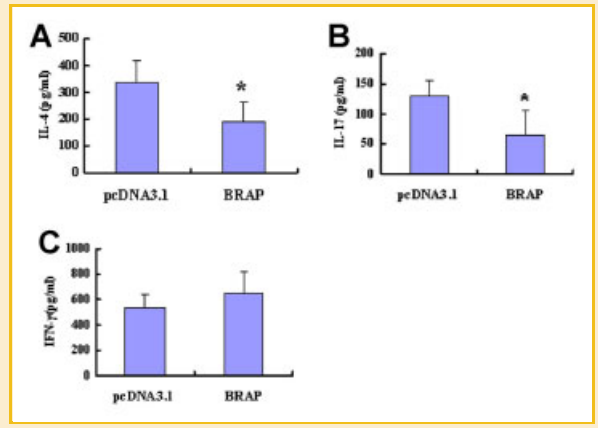

Fig. 6. IL-4 and IL-17, but not IFN-γ secretion is downregulated by BRAP in lymphocytes co-cultured with HBECs. After co-culture of OVA-pretreated lymphocytes with HBECs either transfected with the control plasmid pcDNA3.1(+) or pcDNA3.1(+)/BRAP (BRAP) for 48 h, IL-4 (panel A), IL-17 (panel B), and IFN-γ (panel C) levels were determined in supernatants by ELISA($^{*}P < 0.05$ vs. pcDNA3.1(+) vector control).

hypothesis, we measured the concentrations of IFN-γ, IL-4, and IL-17 in supernatants of the co-culture system since IFN-γ, IL-4, and IL-17 are all typical T cell dependent cytokines that can be used to represent different T cell subpopulation after T cells are activated by antigen presentation [Oei et al., 2004; Nakajima and Hirose, 2010]. Results showed that compared with vector control transfected HBECs, BRAP overexpression in HBECs resulted in inhibition of lymphocyte-dependent secretion of IL-4 and IL-17 (Fig. 6A,B), but had no influence on the secretion of IFN-γ (Fig. 6C).

DISCUSSION

Airway epithelial cells form the first barrier to environmental pollutants and allergens. They play a key role during the protection of lung and the homeostasis of local microenvironment by their structural integrity. Previous investigations suggest that airway epithelial cells may also function as a non-professional antigen-presenting cell (APC) in response to endogenous or exogenous stimuli [Salik et al., 1999; Oei et al., 2004]. In our recent study, we found that HBECs can act as a non-professional APC and they have the ability to present antigens to lymphocytes and to activate lymphocytes in vitro [Liu et al., 2012]. Furthermore, our unpublished data demonstrated that BRS-3 may have impact on the antigen presenting process of HBECs by enhancing the antigen uptaking ability of those cells. Since BRAP is a protein that can interact with BRS-3, we investigated the possible effects of overexpression of BRAP on the antigen presenting ability of HBECs.

Before presenting antigen to T cells, APC must recognize and uptake the antigen first. To explore the ability of BRAP in HBECs on antigen uptake, we incubated HBECs with FITC-OVA and detected the mean fluorescence intensity of the endocytosed antigen at different time points. The results showed a significant decrease of fluorescence intensity in BRAP overexpressing HBECs as compared with control HBECs. These findings indicate that BRAP

overexpression in HBECs significantly reduces the ability of antigen uptake and suggest that BRAP may play a role in the regulation of antigen presenting ability of HBECs.

During an immune response, antigen-presenting cells endocytose foreign antigen, and then display a fragment of the antigen which is bound to a class II MHC molecule on the cell surface. The T cell recognizes and interacts with the antigen-class II MHC molecule complex on the membrane of the APC through T cell receptor (TCR) and is activated by an additional surface co-stimulatory signal on the antigen-presenting cell, leading to activation of the T cell. Therefore, the optimal stimulation and activation of T cells by APC require both T cell receptor engagement and co-stimulation, whereas, in contrast, the absence of co-stimulation may lead to T cell anergy [Bugeon and Dallman, 2000]. Increasing evidence showed that airway epithelial cells not only express major histocompatibility complex (MHC) class I and class II molecules, but also express co-stimulatory molecules such as the B7 family [Kurosawa et al., 2003; Heinecke et al., 2008]. HLA-DR is one of the classical MHC II molecules and has been shown to be present on the surface of HBECs and colocalize with antigen fragment which is processed and presented on the cell surface of HBECs [Oei et al., 2004]. The B7 family is comprised of 7 co-stimulatory molecules, namely B7.1, B7.2, B7-H1, B7-H2, B7-H3, B7-H4, and B7DC [Greenwald et al., 2005] and some of those molecules were found to be upregulated in HBECs when HBECs were stimulated with foreign antigen in our previous study [Liu et al., 2012]. In this study, we showed that overexpression of BRAP, resulted in a changed expression pattern of some of the above surface molecules involved in antigen presentation, for example, an increase of HLA-DR and B7DC expression on the surface of HBECs and a decrease of B7H1 and B7.2 (CD86) expression on the cell membrane. These data provide further evidence for the possible role of BRAP in regulation of antigen presenting process of HBECs. However, the regulatory mechanisms underlying the surface molecule expression upon BRAP overexpression and the internal relationship between those molecules expression and antigen presenting ability of HBECs remain unclear and more work is needed to clarify them.

In this study, we also showed that BRAP overexpressed in HBECs resulted in decreased production of IL-4 by co-cultured lymphocytes, while production of IFN-γ remained unchanged. Once T cells received signals from APC and activated, they proliferate and develop into effector T cells including two major subtypes known as Th1 and Th2 cells, depending on the cytokine profile they produce. Numerous studies have demonstrated that the imbalance of Th1/Th2 cell types plays an important role during inflammatory processes and airway hyperresponsiveness (AHR), whereby a Th2 bias differentiation may promote lung inflammatory responses and AHR [Holgate and Davies, 2009]. Interleukin 4 (IL-4) is usually recognized as a typical Th2 cytokines, while interferon-γ (IFN-γ) is a typical Th1 cytokine [Siwiec et al., 2009]. Therefore, our results indicate that BRAP overexpressing HBECs might have negative regulatory effect on a Th2-biased immune response by inhibiting IL-4 secretion from lymphocytes.

Th17 cells is a subset of IL-17-producing effector T helper cells and IL-17 is reported as a pro-inflammatory cytokine which may provoke neutrophil mobilization and infiltration, promote the secretion of mucus glands, and was also reported to participate in the progress of airway hypersensitivity and airway remodeling [Park and Lee, 2010; Zhao et al., 2010]. Our study demonstrates that BRAP overexpressed in HBECs was followed by decreased IL-17 levels secreted from lymphocytes, indicating that BRAP may restrain airway inflammation and airway hyper-responsiveness by suppressing the Th2 and Th17 immune responses.

In conclusion, our findings suggest that antigen uptake by human bronchial epithelial cells is regulated by BRAP, a novel protein most recently identified as a protein interacting with the orphan G protein-coupled bombesin receptor subtype-3. Overexpression of BRAP may decrease the ability of HBECs to present antigen to T helper lymphocytes and may have potential role in negatively regulating the lymphocyte activation in airway inflammation process. The underlying molecular mechanisms by which BRAP affects the immune functions of HBECs need to be further investigated in future studies.

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