CD40 and IFN-γ dependent T cell activation by human bronchial epithelial cells

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Abstract: We examined whether freshly isolated human bronchial cells (HBEC) and bronchial epithelial cell line/ BEAS-2B cells expressed surface molecules required for APC function. These cells expressed CD40 and ICAM-1, but not B7-1, B7-2 or HLA-DR molecules. Treatment of these cells with IFN-γ resulted in enhanced expression of CD40 and ICAM-1 as well as induction of HLA-DR expression. Th2 cytokines such as IL-4 and IL-5, proinflammatory cytokine of GM-CSF and nonspecific activator endotoxin had no effect on these phenotypic expressions. Functional examinations showed that allogeneic lymphocytes purified from peripheral blood strongly proliferated in response to BEAS-2B cells cultured with IFN-γ, but only weakly compared with those without IFN-γ. When allogeneic lymphocytes were purified to $CD4^+$ cells, the proliferative response against BEAS-2B cells was abolished. Blockade of CD40-CD40L interaction by anti-CD40 antibody also inhibited the proliferation of lymphocytes to BEAS-2B cells, although this treatment showed a minimum effect on the response to allogeneic MNC. Thus, bronchial epithelial cells have the ability to present allogeneic antigens to T cells in both CD40- and IFN-γ- dependent manners under the presence of third party cells that transduce co-stimulatory signals. J. Med. Invest. 48 : 109-117, 2001

Keywords : *bronchial epithelial cells, APC, T cells, CD40, IFN-γ*

INTRODUCTION

Human allergic asthma is characterized by chronic inflammation of the airway. Th2-type cells are thought to be primary initiators of this reaction by producing Th2-type cytokines such as IL-4, IL-5 and IL-13 or by interacting with resident cells in the airway (1, 2). Dendritic cells (DCs) or alveolar macrophage (AM) populations were suggested to be major effectors for the interaction with Th2 cells (3, 4), although other cell types such as fibroblasts or smooth muscle cells may have some role for the interaction with T cells (5, 6). However, there is no sufficient consensus whether the interaction of bronchial epithelial cells with T cells is important for the pathogenesis of airway inflammatory disease. Bronchial epithelial cells produce various inflammatory cytokines including IL-1, GM-CSF, IL-8, RANTES, TNF-α and IL-6 (7-9). Phenotypic examination shows that these cells express adhesion molecules of ICAM-1, VCAM-1, VLA-4, E-selectin (10, 11) and CD40. Furthermore, bronchial epithelial cells exhibit or can be induced to express MHC class II antigens (12, 13) that are essential for the presentation of external antigen. Therefore, bronchial epithelial cells have some characteristics of antigen-presenting cell (APC) that can interact with T cells and can regulate the function of T cells.

The CD40 molecule, as well as the B7 system were shown to be important as co-stimulatory molecules for the activation of T cells (14-18). CD40, a member of the TNF receptor family, is expressed on a variety of cell types such as B lymphocytes, dendritic cells, macrophages, endothelial cells and epithelial cells

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(19-23). The CD40-CD40L interaction was shown to be important for the induction of inflammatory response in the airway (24, 25).

In the present study, we examined whether freshly isolated human bronchial cells (HBEC) and bronchial epithelial cell line (BEAS-2B) cells expressed various surface molecules required for APC function in response to IFN-γ and whether bronchial epithelial cells can induce T cell activation by presenting allogeneic antigens.

MATERIALS AND METHODS

Reagents and Cell lines

Recombinant human IFN-γ (specific activity > 1.0×10^7 U/mg protein) was purchased from Nippon Roche (Tokyo, Japan). Recombinant human GM-CSF (specific activity, 1.7×10^7 U/mg protein) was purchased from Genzyme (Boston, MA). Recombinant human IL-5 was from Suntory Institute for Biomedical Research (Osaka, Japan). Recombinant human IL-4 (specific activity 1.0×10^6 U/mg protein) was kindly provided by Ono Pharmaceutical Co. (Osaka, Japan). Anti-human CD40, anti-human ICAM-1 and anti-human HLA-DR mAbs were purchased from Pharmingen (San Diego, CA). FITC conjugated goat anti-mouse IgG was purchased from Immunotech (Marseille, France). A human bronchial epithelial cell line (BEAS-2B), transformed with SV40 virus, was purchased from the American Type Culture Collection (Rockville, MD). The cells at passages 43-45 were cultured in LHC9/RPMI 1640 medium.

Isolation of Human Bronchial Epithelial cells (HBEC)

Human bronchial epithelial cells were obtained by brushing the bronchial mucosa under bronchoscopy. An intramuscular injection of 0.5 mg of atropine and 25 mg of hydroxyzine was administered 30 min before the examination. Fiberoptic bronchoscopy was always carried out by the same examiner. A fiberoptic bronchoscope (Model 1T20 ; Olympus Optical Co., Tokyo, Japan) was inserted into the trachea. Local anesthesia of the bronchial mucosa was achieved with 2% lidocaine. Brushing for collection of bronchial epithelial cells was performed in a standard manner before procedures such as bronchoalveolar lavage, transbronchial lung biopsy, and bronchial biopsy for clinical purposes. The disposable brush (Model BC-15C ; Olympus) was introduced via the bronchoscope into subsegmental bronchi of the left B5a segment and rubbed against the epithelial surface by 10 gentle upward and downward strokes. In cases of bronchial carcinoma with a tumor in the left bronchus, the right subsegmental bronchus was brushed. The cells obtained by brushing were collected in 50 ml polypropylene tubes containing 20 ml RPMI 1640 with gentamycin by shaking the brush in the medium. The cells were then washed twice by centrifugation at 400×g at 4°C for 10 min for use in subsequent experiments. All patients gave informed consent to participate in the study.

Culture of HBEC

The rinsed pellet of HBEC obtained by brushing was resuspended in LHC9/RPMI 1640 medium. The cells were plated in tissue culture plates with 16-mm/diameter wells coated with a gel of type I collagen (Vitrogen100 ; Celtrix, Santa Clara, CA), and incubated at 37°C, under 5% CO_2 in air for 7 to 10 days. The medium was changed after 24 hr and then every other day. When the cells reached confluency, they were passaged in 48-well tissue plates. At the same time, they were confirmed to be entirely epithelial cells by their positive staining by the avidin-biotin complex method (ABC kit ; VECTOR Lab., Burlingame, CA) with monoclonal murine anti-human cytokeratin antibody (MAK-6 ; Triton, Alameda, CA). No cells reacted with monoclonal murine anti-swine vimentin antibody (DAKO-Vimentin ; DAKO, Santa Barbara, CA), which was demonstrated to stain fibroblast IMR-90 cells provided by Tokyo Metropolitan Institute of Gerontology (Tokyo, Japan). At the time of semiconfluency, cells were used in experiments.

Isolation and purification of lymphocytes and $CD4^+$ cells

Leukocyte concentrates from healthy donors were separated into peripheral blood mononuclear cells (PBMC) by density gradient centrifugation in lymphocyte separation medium. Subsequently, PBMC were separated into lymphocytes and monocytes by counterflow centrifugal elutriation in a Beckman JE-5.0 rotor (Beckman Instruments, Inc., Fullerton, CA). The lymphocyte-rich fraction was collected at flow rate of 12 to 16 ml/min at 2000 rpm. The purity of the lymphocyte fraction was determined by morphologic examination and was more than 99%. $CD4^+$ cells were isolated from this lymphoid fraction by incubation with CD4-specific Dynabeads, followed by Detatchabead treatment, as indicated by the manufacture (Dynabeads, Oslo, Norway). This procedure yielded a population of > 98% $CD4^+$ cells, as determined by FACS analysis.

Analysis by flow microfluorometry

HBEC or BEAS-2B cells were cultured for 3 days in the presence or absence of IFN-γ, GM-CSF, IL-4, IL-5 and LPS. The cells were harvested by 0.125% tripsin containing 0.02% EDTA. The single cell suspensions were incubated with mAb against CD40, HLA-DR and ICAM-1 for 30 min at 4°C in PBS containing 1% FBS. Then, cells were washed twice with PBS containing 1% FBS. The stained cells were measured by FACStation™ (Becton Dickinson, Mountain View, CA) using CellQuest software.

Measurement of T cell proliferation

Purified lymphocytes or $CD4^+$ cells (1×10^5/well) were cocultured with BEAS-2B or allogeneic mononuclear cells (MNC) for 4 days under the presence of a suboptimal dose of Con A (5 μg/ml) at a ratio of 1 : 5 in LHC9/RPMI 1640 containing 10% FBS in a 96-well microplate at 37°C under 5% CO_2. BEAS-2B cells used for stimulation were cultured with or without IFN-γ (100 U/ml) for 3 days before the experiment. Allogeneic mononuclear cells were prepared from blood of healthy donors by density gradient centrifugation in lymphocyte separation medium. Stimulator cells were pretreated with 25 μg/ml of MMC for 30 min. After 4 days of incubation, 1 μCi/well of ^{3}H-thymidine (Amersham Arlinton Height, IL) was added and further incubated for 20 hr. The cells were harvested on a glass filter with a cell harvester (Labo Mash, Tokyo, Japan). The incorporation of ^{3}H-thymidine was examined in a liquid scintillation counter (LSC-3500 ; Aloka, Tokyo, Japan).

RESULTS

Expression of CD40 molecules on bronchial epithelial cells and the enhancement by IFN-γ

CD40 is an important co-stimulatory molecule which is responsible for initiation of the inflammatory reaction. We then evaluated the expression of CD40 on BEAS-2B cells and HBEC by culturing with or without IFN-γ for 3 days. The expression of CD40 was detectable, but the expression level was low in BEAS-2B and HBEC cells (Fig. 1). Treatment with IFN-γ enhanced the expression of CD40 about 5-fold in mean fluorescence intensity in these cells. This enhancement was dose-dependent and the expression reached maximum at 2 or 3 days of incubation (data not shown). In the subsequent experiments, we used the maximum level of IFN-γ (100 U/ml).

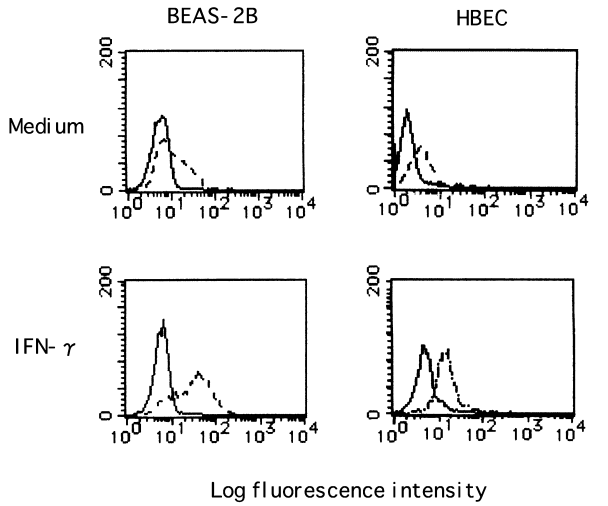

Fig. 1. Expression of CD40 on BEAS-2B cells and HBEC. BEAS-2B cells and HBEC were cultured for 3 days with or without IFN-γ (100 U/ml). These cells were stained with anti-CD40 (thin line) or only with second antibody (broken line). The expression of CD40 was examined by FACStation™.

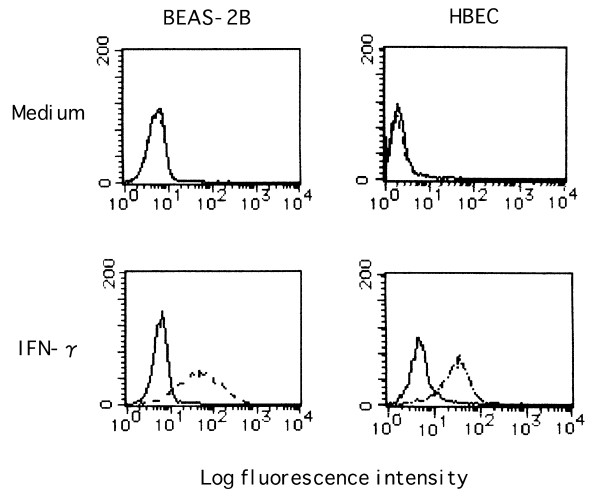

Fig. 2. Induction of HLA-DR expression on BEAS-2B cells and HBEC by IFN-γ. BEAS-2B cells and HBEC were cultured for 3 days with or without IFN-γ (100 U/ml). These cells were stained with anti-HLA-DR (broken line) or only with second antibody (thin line). The expression of HLA-DR was examined by FACStation™.

Induction of HLA-DR expression on bronchial epithelial cells by IFN-γ

Expression of class II molecules is necessary for the presentation of external antigens. To ascertain the ability of HBEC or BEAS-2B cells to express class II antigen, these cells were cultured with IFN-γ for 3 days and the expression of HLA-DR was assessed. Although HBEC and BEAS-2 B cells did not express HLA-DR in the resting condition without IFN-γ, both of these cells expressed significant HLA-DR after IFN-γ treatment (Fig. 2). HLA-DR expression was induced

dose-dependently and reached maximum after 3 to 4 days of incubation (data not shown).

Expression of co-stimulatory molecules other than CD40 on bronchial epithelial cells and the regulation by IFN-γ

We examined the expression of the co-stimulatory molecules other than CD40 in HBEC and BEAS-2B cells. ICAM-1 expression, but no expression of B 7-1 or B7-2 was detected under the resting condition (Fig. 3). In a parallel experiment, treatment with IFN-γ strongly enhanced the expression of ICAM-1 especially in HBEC up to 100-fold in mean fluorescence intensity, but no induction of B7-1 or B7-2 was obsereved. These changes in adhesion molecule expression were similar in both of these cells.

Selective requirement of IFN-γ for the expression of HLA-DR antigen on BEAS-2B cells

The inflammatory reaction of asthma was often seen in Th2 cell dominant conditions (1, 2) Therefore, we examined whether Th2 cytokines or other proinflammatory cytokine such as IL-4, IL-5 and GM-CSF could induce the expression of HLA-DR on BEAS-2B cells. However, all the cytokines employed in this experiment except IFN-γ did not cause HLA-DR induction on BEAS-2B cells (Fig. 4). Nonspecific strong stimulator LPS also did not induce the expression of HLA-DR. Therefore, IFN-γ appeared to be a selective inducer for HLA-DR expression on human bronchial epithelial cells.

Allogeneic lymphocytes, but not purified $CD4^+$ cells proliferated in response to BEAS-2B cells stimulated with IFN-γ

Lymphocytes (T cells and B cells) or $CD4^+$ cells purified from peripheral blood MNC were co-cultured for 5 days with BEAS-2B cells pretreated with or without IFN-γ or allogeneic MNC. Allogeneic lymphocytes strongly proliferated in response to BEAS-2B cells treated with IFN-γ. In contrast, untreated BEAS-2B cells induced only minor proliferation of lymphocytes (Fig. 5). The enhancement of T cell proliferation by BEAS-2B cells cultured with IFN-γ was attributable to the expression of class II antigen and the enhanced

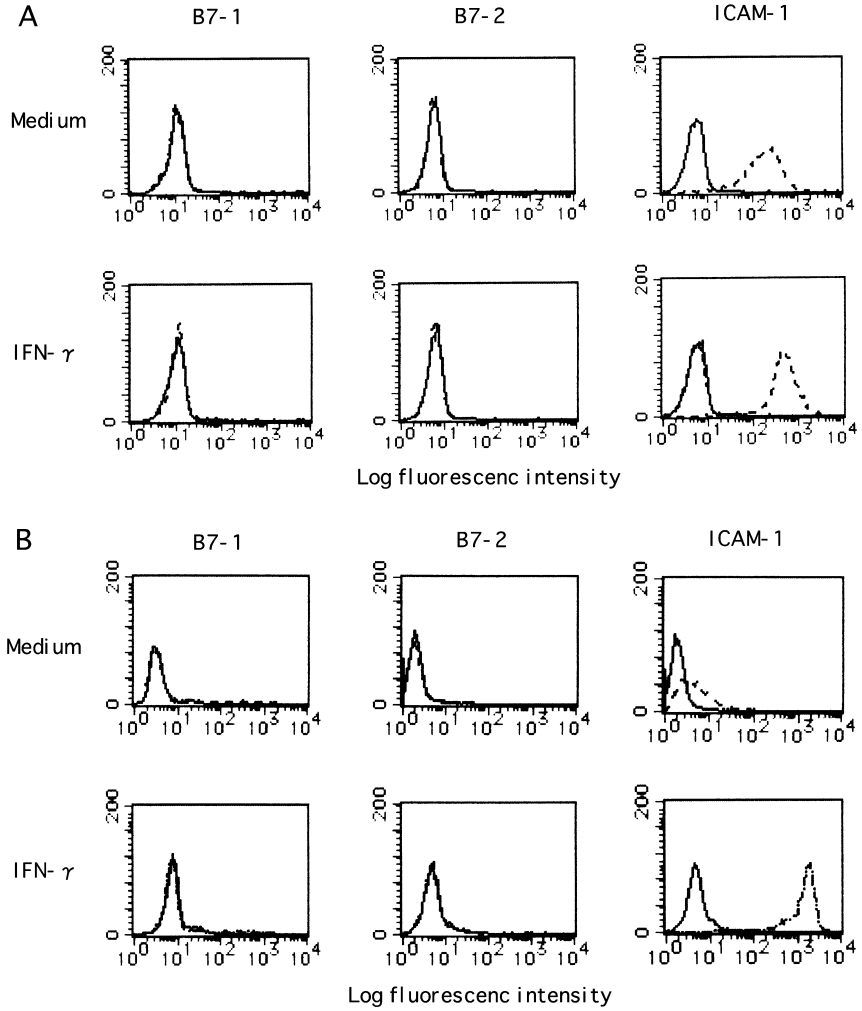

Fig. 3. Exprssion of B7-1, B7-2 and ICAM-1 on BEAS-2B cells and HBEC under the presence or absence of IFN-γ. BEAS-2B cells (A) and HBEC (B) were cultured for 3 days with or without IFN-γ (100 U/ml). These cells were stained with anti-B7-1, anti-B7-2, anti-ICAM-1 (broken line) or only with second antibody (thin line). The expression of HLA-DR was examined by FACStation™.

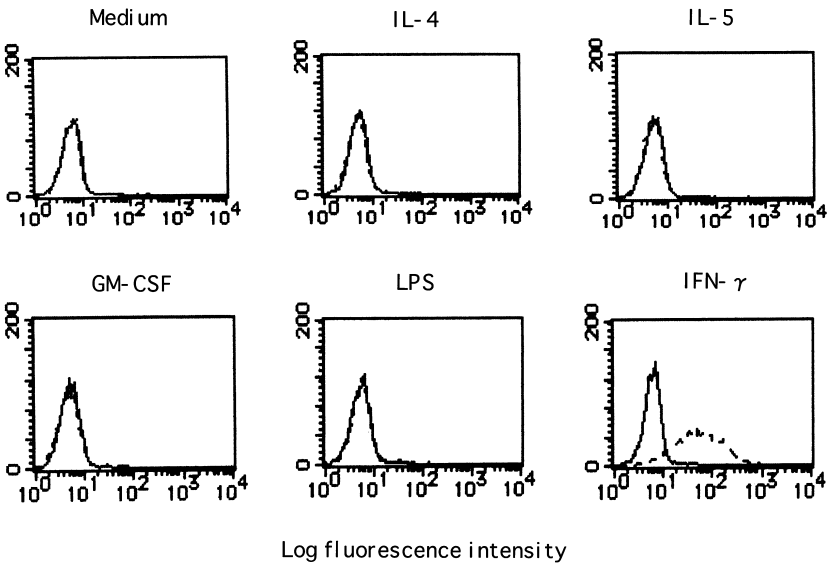

Fig. 4. No induction of HLA-DR expression by IL-4, IL-5, GM-CSF and LPS on BEAS-2B cells. BEAS-2B cells were cultured for 3 days with medium, IL-4 (100 U/ml), IL-5 (100 U/ml), GM-CSF (100 U/ml), LPS (5 μg/ml), or IFN-γ (100U/ml). After harvesting, these cells were stained with anti-HLA-DR (broken line) or only with second antibody (thin line). The expression of HLA-DR was examined by FACStation™.

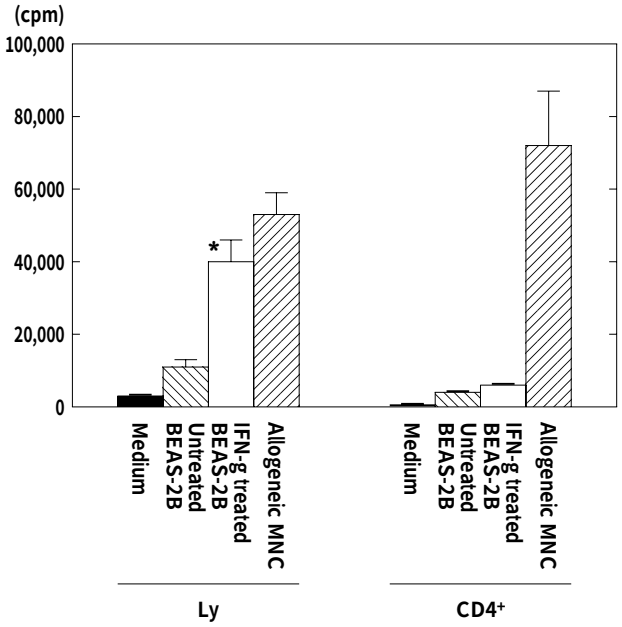

Fig. 5. Proliferation response of T cells to the stimulation with BEAS-2B cells. BESA-2B cells were precultured with or without IFN-γ (100 U/ml) for 3 days. $CD4^+$ cells or lymphocytes (Ly) purified from peripheral blood were cultured with IFN-γ treated BEAS2B, untreated BEAS-2 B or allogeneic MNC for 5 days. T cell proliferation was quantitated by ^3H-thymidine incorporation (see Materials and Methods). *, $p \leqq 0.01$, compared with untreated BEAS-2B

expression of co-stimulatory molecules such as CD40 or ICAM-1. In a parallel experiment, when purified $CD4^+$ cells were used as responder cells, proliferation of T cells to IFN-γ-treated BEAS-2B cells was almost completely abolished. Thus, the co-stimulatory signal delivered from non-$CD4^+$ cells in the lymphoid population is required for the induction of T cell proliferation.

Effect of anti-CD40 mAb on the proliferation of T cells to BEAS-2B cells

The CD40 molecule was constantly expressed on bronchial epithelial cells. We next examined whether blockade of the CD40-CD40L interaction by anti-CD40 mAb affected the allogeneic reaction of lymphocytes to bronchial epithelial cells in terms of T cell activation. Although T cell proliferation to allogeneic MNC was only partially affected by treatment with anti-CD 40 mAb, T cell proliferation to IFN-γ-treated BEAS-2B cells was strongly suppressed (Fig. 6). The inhibitory effect of anti-CD40 mAb was also found in the T cell response to non-treated BEAS-2B cells. Thus, the CD40 molecule appeared to be involved in the class I response as well as class II response. The CD40 molecule may be essential for bronchial epithelial cells rather than for professional APC to interact with T cells, since bronchial epithelial cells did not express other co-stimulatory molecules of B7-1 and B7-2.

DISCUSSION

There is accumulating evidence that Th2 cells are primary initiators of asthmatic inflammatory reactions (1, 2). DCs or AM were suggested to be the main populations for presentation of external antigen to T cells in the airway (3, 4). The interaction of T cells with other resident cells such as smooth muscle cells or fibroblast cells was also suggested to be involved in the pathogenesis of pulmonary inflammation (5, 6). However, the importance of the interaction between bronchial epithelial cells and T cells has not been sufficiently evaluated.

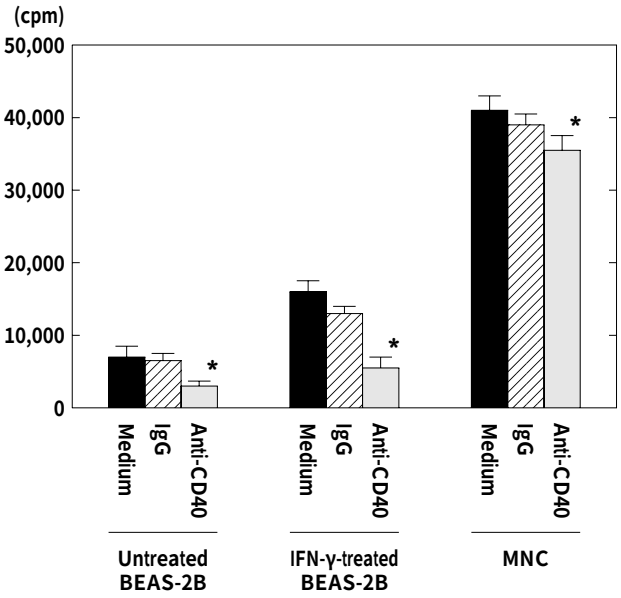

Fig. 6. Inhibition of T cell proliferation by anti-CD40 treatment. BEAS-2B cells were precultured with or without IFN-γ (100 U/ml) for 3 days. Lymphocytes purified from peripheral blood were cultured with IFN-γ treated BEAS-2B, untreated BEAS-2B or MNC for 5 days under the presence of anit-CD40 or control IgG. T cell proliferation was quantitated by ^{3}H-thymidine incorporation (see Materials and Methods). *, $p \leqq 0.05$, compared with the respective IgG treatments.

In the present study, to explore the possibility of the APC function of bronchial epithelial cells, we examined whether human bronchial epithelial cells activate T cells by presenting allogeneic antigen. First, the present study clearly showed that IFN-γ induced the expression of HLA-DR and augmented the expression of ICAM-1 and CD40 on BEAS-2B and HBEC cells. Moreover, functional analysis showed that BEAS-2B cells stimulated the proliferation of allogeneic lymphocytes (mixture of T and B cells) when BEAS-2B cells were cultured with IFN-γ. Thus, BEAS-2B cells have the ability to induce a class II response under the presence of IFN-γ. However, untreated BEAS-2B cells have a minor effect on T cell proliferation probably due to the response to class I antigen only.

When purified CD4$^+$ T cells were used as a responder, the stimulatory effect of BEAS-2B cells was almost completely abolished compared with that of allogeneic MNC. Here, we showed no induction of B7-1 or B7-2 expression by IFN-γ in HBEC and BEAS-2B cells. Furthermore, in a separate experiment, we found that these two molecules in BEAS-2B cells were also not induced by CD40 stimulation (data not shown). Previous studies showed that resting B cells express CD40 (19) and B7-2 but not B7-1 (26). These findings raised one possibility that B cells in the lymphoid populations could deliver co-stimulatory signals for activation of T cells, although there was a controversial study where CD40 expressed on B cells was rather inhibitory in the interaction of T cells and APC (27).

Blockade of the CD40-CD40L interaction by CD40 mAb resulted in inhibition of the T cell proliferation induced by BEAS-2B cells independent of whether these cells were treated with IFN-γ. Taken together with the findings using purified CD4$^+$ T cells, signaling from CD40 is essential for the activation of CD4$^+$ T cells. The CD40L molecule is also expressed on CD8$^+$ T cells (28, 29) as well as on CD4$^+$ T cells and is involved in the immune response of the intestine (30). Anti-CD40 treatment may also inhibit the response of CD8$^+$ T cells to untreated BEAS-2B cells.

In the mucosal area of the intestine, the immune response is regulated through antigen presentation by mucosal epithelial cells (31-33). The mechanism of interaction between intestinal epithelial cells and T cells is well documented (34, 35). When external antigens are presented via the mucosal (oral or nasal) route, no T cell response is induced (36-38). Co-stimulatory molecules such as B7 and CD40 are involved in this regulation (39, 40). Moreover, there are studies showing that IFN-γ is important for the induction of mucosal tolerance (37, 41). Consistent with these previous findings, we observed that the interaction between bronchial epithelial cells and T cells was also CD 40 and IFN-γ dependent. This interaction may be important for the control of the overreaction of T cells in the airway through the mimicked mechanism of activation induced cell death (42-44). The potentials of APC function by bronchial epithelial cells was induced selectively by IFN-γ, but not by Th2-type cytokines of IL-4 and IL-5, proinflammatory cytokine of GM-CSF and non-specific stimulator LPS. There are several studies showing that increased production of IFN-γ reduced the allergic condition including bronchial asthma (45-47). It is possible that interaction between epithelial cells and T cells is insufficient in asthmatic patients due to the Th2 dominant conditions.

In conclusion, the present findings indicated that bronchial epithelial cells have characteristic features of APC in terms of phenotype and function, and that APC function of bronchial epithelial cells could be mediated by IFN-γ and CD40 dependent mechanism under the presence of third party cells. Further experiments are required to confirm whether naive bronchial epithelial cells can function as APC.

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