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Biochemical and Biophysical Research Communications 330 (2005) 263–270
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Expression of B7-H1 and B7-DC on the airway epithelium is enhanced by double-stranded RNA

Miyuki Tsuda [a], Koichiro Matsumoto [a], Hiromasa Inoue [a,*], Mikiko Matsumura [a], Takako Nakano [a], Akio Mori [b], Miyuki Azuma [c], Yoichi Nakanishi [a]

[a] *Research Institute for Diseases of the Chest, Graduate School of Medical Sciences, Kyushu University, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*
[b] *Clinical Research Center for Allergy and Rheumatology, National Hospital Organization Sagamihara National Hospital, Sakuradai, Sagamihara, Kanagawa 228-8522, Japan*
[c] *Department of Molecular Immunology, Graduate School, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo 113-8549, Japan*

Received 18 February 2005

Abstract

Viral infection in the airway provokes various immune responses, including Th1 and Th2 responses, which are partly initiated by double-stranded RNA (dsRNA), a viral product for its replication. B7-H1 (PD-L1) and B7-DC (PD-L2) are B7-family molecules that bind to programmed death-1 (PD-1) on lymphocytes and are implicated in peripheral tolerance. We investigated the effect of dsRNA on the expression of B7-H1 and B7-DC on airway epithelial cell lines. B7-H1 and B7-DC were constitutively expressed on the cells, and their expression was profoundly upregulated by stimulation with an analog of viral dsRNA, polyinosinic–polycytidylic acid. B7-H1 and B7-DC were also upregulated by stimulation with IFN-γ, IL-13, and the supernatant from T cell clones. A relatively high concentration of dexamethasone (1 μM) was required to suppress the upregulation of B7-H1 or B7-DC. These results suggest that epithelial B7-H1 and B7-DC play a role in virus-associated immune responses in the airways.

Keywords: Innate immunity; Double-stranded RNA; IFN-γ; IL-13; Programmed death-1; BEAS-2B; T cell clone; Glucocorticoid

The elucidation of epithelial cell-to-virus interactions, particularly, in the context of immune regulation, has been an important research target in the area of airway diseases [1]. The rhinovirus, respiratory syncytial virus (RSV), influenza virus, and parainfluenza virus are known to exacerbate asthma and chronic obstructive pulmonary diseases (COPD). These viruses synthesize double-stranded RNA (dsRNA) for their replication. Recently, dsRNA was identified as a natural ligand for Toll-like receptor 3 (TLR3) [2], and it was shown that virus-infected epithelial cells secrete several chemokines in response to dsRNA via TLR3, dsRNA-dependent protein kinase (PKR), and mitogen-activated protein kinases [3–5] Although these responses are associated with innate immunity against viral invasion, the following process may frequently lead to the exacerbation of underlying diseases.

Virus-infected cells express the virus-associated antigens on the major histocompatibility complex (MHC) class I and await cytolysis by cytotoxic/killer T cells, a mechanism which prevents the further spread of the virus [6,7]. On the other hand, viral species have developed escape mechanisms from the host immune system [8], including interruptions of the infected cell-to-T cell responses. When antigen-specific T cells recognize the MHC-class I/II-antigen peptide complex on their counterparts, the following T cell responses are critically affected by the simultaneous signaling operated through costimulatory molecules. B7-1 (CD80) and B7-2 (CD86)

* Corresponding author. Fax: +81 92 642 5389.
E-mail address: inoue@kokyu.med.kyushu-u.ac.jp (H. Inoue).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.02.161

are well-characterized costimulatory molecules expressed on professional antigen-presenting cells (APCs). These molecules are upregulated by various stimuli, such as LPS and dsRNA [9]. During the last decade, an array of molecules with homologous sequences to B7-1 and B7-2 have been identified, including B7-H1 (PD-L1), B7-H2 (inducible costimulator ligand; ICOSL), B7-H3, B7-H4 (B7S1, $B7_X$), and B7-DC (PD-L2).

B7-H1 and B7-DC are ligands for programmed death-1 (PD-1), which is expressed on activated T and B cells [10–13]. Colligation of PD-1 and the T cell receptor (TCR) leads to rapid phosphorylation of SHP-2, a phosphatase suggested to inhibit T cell activation. Indeed, various experimental disease models using PD-1-deficient mice or neutralizing antibodies against B7-H1, B7-DC, or PD-1 suggested that B7-H1 and B7-DC have a crucial role in the regulation of immune responses in the periphery [14–18]. More interestingly, B7-H1 and B7-DC are expressed not only on professional APCs but also on tissue structural cells, including endothelial cells, keratinocytes, and several tumor cells [11,19–21]. These findings imply that structural cells might actively contribute to the maintenance of various immune responses. Thus, we sought to assess the expression of B7-H1 and B7-DC on human airway epithelial cells and investigated whether virus-associated compounds affect their expression.

Materials and methods

Culture of airway epithelial cells. The BEAS-2B and the 16HBE, both of which are SV40-transformed human bronchial epithelial cell lines, and the A549, a human type II alveolar epithelial cell line, were cultured in DMEM/F12 containing 10% FBS, 100 U/ml penicillin, and 100 ng/ml streptomycin (Invitrogen, Tokyo, Japan) at 37 °C with 5% CO_2 in humidified air.

Stimulation of airway epithelial cells. When cells reached 90% confluence, they were stimulated with various concentrations of polyinosinic–polycytidylic acid (referred to as dsRNA; Sigma–Aldrich, St. Louis, MO), polycytidylic acid (referred to as single-stranded RNA, ssRNA; Sigma–Aldrich), human recombinant IFN-γ (Techne, Minneapolis, MN), IL-13 (Daiichi Pharmaceutical, Tokyo, Japan) or both of the cytokines for 24 h. In the study of dsRNA, several samples were treated with 7.5 μg/ml of rabbit anti-human IFN-β antibody (Chemicon International, Temecula, CA) for neutralization of IFN-β. Some dsRNA was pretreated with an excessive amount of RNase (Qiagen, Tokyo, Japan) for 30 min to determine whether the biological effect of dsRNA in this study is derived from its ribonucleic acid structure. Several samples were incubated with ranging concentrations of dexamethasone (Banyu Pharmaceutical, Tokyo, Japan) 30 min before stimulation. After stimulation, the supernatants of the cell culture were collected for the quantification of chemokines by ELISA. Remaining cells were treated with 0.02% trypsin/EDTA (Sigma–Aldrich) in HBSS (Invitrogen), washed three times with Ca^{2+}–Mg^{2+}-free HBSS, and further treated with Ca^{2+}–Mg^{2+}-free HBSS containing 0.02% EDTA without trypsin for 20 min. Cells were harvested by repeated pipetting and then processed for flow cytometry.

Stimulation of airway epithelial cells with culture supernatant of T cell clones. Der f 2-specific human T cell clones were generated from peripheral blood mononuclear cells (PBMCs) of atopic asthmatic donors by antigenic stimulation, which was followed by the limiting dilution methods as described previously [22]. Briefly, PBMCs (2×10^6/ml) were cultured in an AIM-V medium (Life Technologies, Gaithersburg, MD) with 1 μg/ml recombinant Der f 2 protein (Asahi Food and Healthcare, Tokyo, Japan). After 10 days of culture, 10^2 to 10^4 live non-adherent cells were cultured in 96-well round-bottomed culture plates (Nunc, Roskilde, Denmark) with antigen and 2500 rad-irradiated autologous PBMCs (5×10^4 cells). A fresh medium containing 10 U/ml recombinant IL-2 (kindly provided by Shionogi Pharmaceutical, Osaka, Japan) was added once per week. When fewer than 1 of 10 wells contained proliferating cells, the resulting cell lines were considered to have originated from a single clone. To ensure their clonality, these T cells were further subcloned by limiting the dilution using irradiated autologous PBMCs and antigen. After 10–14 days, expanding cultures were transferred to 24-well culture plates (BD Biosciences, San Diego, CA). These clones were maintained by antigenic stimulation with irradiated autologous PBMCs (2×10^6/ml) and antigen every 2–3 week.

T cell clones were harvested at least 10 days after the last antigenic stimulation and suspended in an RPMI-1640 medium with 10% FBS and penicillin/streptomycin (Invitrogen). Cells (5×10^6/ml) were cultured with or without 10-μg/ml plate-bound anti-human CD3 mAb (OKT3, Ortho, Raritan, NJ) in 6-well culture plates (Greiner Japan, Tokyo, Japan) for 36 h, and their supernatants were then collected. An aliquot of the supernatant was used for the quantification of IFN-γ, IL-5, IL-13, and TNF-α by ELISA. When BEAS-2B cells reached 90% confluence, their culture medium was carefully replaced with the supernatant of a T cell clone, incubated for 24 h, and then processed for flow cytometry.

Flow cytometric analysis. The mAbs used for flow cytometry were fluorescein isothiocyanate (FITC)-labeled anti-human B7-1 mAb (L307.4, BD Biosciences), FITC-labeled anti-human B7-2 mAb (2331, BD Biosciences), biotinylated anti-human B7-H1 mAb (MIH1), and biotinylated anti-human B7-DC mAb (MIH18). MIH1 and MIH18 were generated as described previously [23]. For each analysis, 5×10^5 BEAS-2B cells were incubated in 100 μl of phosphate-buffered saline (PBS) with 0.5% BSA and 0.02% NaN_3 (Sigma–Aldrich) containing each mAb at room temperature for 30 min. The samples with biotinylated mAbs were washed and suspended in phycoerythrin (PE)-labeled streptavidin (BD Biosciences) for 20 min. After washing with PBS/0.5% BSA/0.02% NaN_3, the cells were fixed with 4% paraformaldehyde (medium A; Caltag Laboratories, Burlingame, CA) for 20 min. Fixed cells were washed again and then processed for flow cytometric analysis using a FACSCalibur flow cytometer and CELLQuest software (BD Biosciences). Ten thousand events were acquired in a list mode with debris excluded by the forward-scatter threshold. The mean fluorescence intensity (MFI) was compared with control staining using an irrelevant isotype-matched mouse mAb.

Quantification of chemokine and cytokine. The levels of eotaxin, regulated on activation, normal T cells expressed and secreted (RANTES), IFN-γ, IL-5, IL-13, and TNF-α in the culture supernatant were measured using an ELISA kit (Biosource International, Camarillo, CA). The level of IFN-β in the supernatant was measured using an ELISA kit (Biomedical laboratories, Picataway, NJ).

Data analysis. Values were expressed as means ± SEM. The data of flow cytometry are shown by histograms or MFI. Differences among groups were analyzed using unpaired t tests or an ANOVA together with a post hoc Bonferroni analysis. A value of $p < 0.05$ was considered significant.

Results

Expression of B7-H1 and B7-DC on unstimulated cells

The flow cytometric study showed that BEAS-2B cells spontaneously expressed B7-H1 and B7-DC on