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ICOS promotes group 2 innate lymphoid cell activation in lungs

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ABSTRACT

Group 2 innate lymphoid cells (ILC2s) are newly identified, potent producers of type 2 cytokines, such as IL-5 and IL-13, and contribute to the development of allergic lung inflammation induced by cysteine proteases. Although it has been shown that inducible costimulator (ICOS), a costimulatory molecule, is expressed on ILC2s, the role of ICOS in ILC2 responses is largely unknown. In the present study, we investigated whether the interaction of ICOS with its ligand B7-related protein-1 (B7RP-1) can promote ILC2 activation. Cytokine production in ILC2s purified from mouse lungs was significantly increased by coculture with B7RP-1-transfected cells, and increased cytokine production was inhibited by monoclonal antibody-mediated blocking of the ICOS/B7RP-1 interaction. ILC2 expansion and eosinophil influx induced by papain, a cysteine protease antigen, in mouse lungs were significantly abrogated by blocking the ICOS/B7RP-1 interaction. Dendritic cells (DCs) in the lungs expressed B7RP-1 and the number of DCs markedly increased with papain administration. B7RP-1 expression on lung DCs was reduced after papain administration. This downregulation of B7RP-1 expression may be an indication of ICOS/B7RP-1 binding. These results indicate that ILC2s might interact with B7RP-1-expressing DCs in allergic inflammatory lung, and ICOS signaling can positively regulate the protease allergen-induced ILC2 activation followed by eosinophil infiltration into the lungs.

1. Introduction

Allergic asthma is characterized by chronic airway inflammation with pronounced eosinophil infiltration, mucus overproduction, and airway hypersensitivity. Although a variety of cell types are involved in allergic inflammation, there is substantial evidence that infiltration of Th2 cells and increased levels of type 2 cytokines, including IL-4, IL-5, and IL-13, are common in the lungs of patients with allergic asthma [1]. Thus, Th2 cells have long been thought to be the primary source of type 2 cytokines [2]. In contrast, subsequent studies revealed that novel innate lymphocytes, called group 2 innate lymphoid cells (ILC2s), potently produce type 2 cytokines in response to epithelial-derived cytokines, such as IL-33 [3–7]. Protease allergens, such as papain, which is a plant-derived cysteine protease, induce the production of these cytokines in epithelial cells [8]. Intranasal administration of papain or IL-33 induced lung eosinophilia and mucus overproduction in recombination activating gene (*Rag*)$^{-/-}$ mice that lack T/B cells [9,10]. Treatment of lung explants with papain increased the production of IL-33 from stromal cells and that of IL-5 and IL-13 by ILC2s [6]. Moreover, papain did not induce eosinophilic lung inflammation in *Il33*$^{-/-}$ or *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice that lack ILC2s [6,10]. Consequently, it appears that ILC2s are capable of inducing allergic lung inflammation by producing type 2 cytokines.

Optimal T cell activation requires two signals: one produced by the engagement of T cell receptors with antigen/major histocompatibility complexes and the other is the costimulatory signal delivered by the interaction of receptors on T cells and their corresponding ligands on antigen-presenting cells (APCs) [11]. The interaction of B7/CD28 family members plays a major role in providing costimulatory signals for T cells [12]. Inducible costimulator (ICOS), a member of CD28 receptor family, is expressed on activated T cells, and its ligand B7-related protein-1 (B7RP-1; also known as B7h, B7-H2, GL50, and ICOSL) is constitutively

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expressed on APCs, including dendritic cells (DCs) [12]. ICOS ligation by B7RP-1 enhances T cell differentiation and effector function [12]. In addition, anti-ICOS monoclonal antibody (mAb)-treated or ICOS-deficient mice showed the attenuated Th2 cell responses and eosinophil accumulation in ovalbumin-induced allergic lung inflammation [13–15]. Thus, ICOS is thought to play a crucial role in Th2 cell responses during the development of allergic lung inflammation. It has been shown that ICOS is also expressed on ILC2s and is a useful target for identifying ILC2s [16]. However, the role of ICOS in ILC2 responses is largely unknown. In this study, we investigated whether ICOS signaling can regulate ILC2 activation.

2. Materials and methods

2.1. Mice

Male C57BL/6 mice were purchased from Charles River Laboratories (Kanagawa, Japan). C.B-17/Icr-scid/scid Jcl (SCID) mice and C.B-17/Icr-+/+Jcl control mice were obtained from Crea (Tokyo, Japan). All mice were used at 6–10 weeks of age and kept under specific pathogen-free conditions during the experiments. All animal experiments were approved by Juntendo University Animal Experimental Ethics Committee.

2.2. Airway inflammation

Mice were intranasally injected with 30 μg papain (Merck Millipore, Darmstadt, Germany) in 40 μl of PBS or PBS alone on 3 consecutive days. Some groups of mice were intraperitoneally administered with 300 μg of anti-B7RP-1 mAb (HK5.3, rat IgG2a) [17] or control rat IgG2a 4 h before each papain injection. At 24 h after the last papain instillation, lungs were collected for subsequent assays.

2.3. Lung cell preparation

Lungs obtained from mice were minced with scissors and incubated in RPMI1640 medium (supplemented with 10 mM HEPES, 2 mM L-glutamine, 0.1 mg/ml penicillin/streptomycin, 50 μM 2-ME) containing 50 μg/ml Liberase TM (Roche, Basel, Switzerland) and 20 μg/ml DNase I (Roche) for 45 min at 37 °C. Digested tissues were further incubated for 5 min in the presence of 5 mM EDTA (Dojindo Laboratories, Kumamoto, Japan) and passed through a 70-μm cell strainer (BD Biosciences, San Jose, CA, USA). The cells were used for sorting or flow cytometry after lysis of red blood cells.

2.4. Flow cytometry

Cells were pre-incubated with unlabeled anti-CD16/32 mAb (2.4G2) to avoid non-specific binding of antibodies to FcγR. Cells were then incubated with the antibodies listed in Supplemental Table S1. Biotin-B7-H3 (MJ18), biotin-GITR (MIH44), biotin-TIM-1 (RMT1-4), biotin-TIM-2 (RMT2-26), biotin-TIM-3 (RMT3-23), biotin-TIM-4 (RMT4-53), and biotin-4-1BBL (TKS-1) were from our laboratory [18–23]. Cells incubated with biotinylated mAb were stained with PE-labeled streptavidin (eBioscience, San Diego, CA, USA). Stained cells were analyzed by BD LSRFortessa (BD Biosciences), and data were processed by FlowJo Version 7.6.5 software (FlowJo, Ashland, OR, USA). Live cells were gated by forward- and side-scatter profiles and 7-AAD (TONBO Biosciences, San Diego, CA, USA) exclusion.

2.5. ILC2 stimulation in vitro

Lineage$^-$ ($CD3\varepsilon^-CD11b^-CD11c^-CD19^-Gr\text{-}1^-TER\text{-}119^-$) and $CD90.2^+CD25^+CD127^+$ ILC2s were sorted from lung cells by JSAN cell sorter (Bay Bioscience, Kobe, Japan). Purified ILC2s (1×10^3 cells/well) were cultured with mitomycin C (Nacalai Tesque, Kyoto, Japan)-treated B7RP-1/P815 or P815 parent cells (1×10^3 cells/well) in the presence of 50 U/ml recombinant human IL-2 (Shionogi, Osaka, Japan), 10 ng/ml recombinant mouse IL-33 (R and D Systems, Minneapolis, MN, USA), anti-B7RP-1 mAb (HK5.3), anti-ICOS mAb (7E.17G9, rat IgG2b, BioLegend), or control rat IgG (20 μg/ml). To determine cytokine production, supernatants were collected after 7 days and assayed for IL-5 by enzyme-linked immunosorbent assay using OptEIA kit (BD Biosciences) and IL-13 using a Ready-SET-Go! Kit (eBioscience), according to the manufacturer's instructions.

2.6. Statistical analysis

Statistical analyses were performed by unpaired Student's *t*-tests. Results are expressed as mean ± SD or SEM, as described in each figure legend. $p < 0.05$ was considered significant.

3. Results

3.1. Expression of costimulatory molecules on lung ILC2s

ILC2s reside in the lung tissue of naïve mice, and intranasal administration of papain rapidly induced the activation of lung ILC2s [6]. We first obtained lung cells from PBS- or papain-treated mice and investigated ICOS expression on resting and activated lung ILC2s by flow cytometry. As shown in Fig. 1A, lung ILC2s were identified as Lineage$^-$ (CD3ε, CD4, CD8α, CD11b, CD11c, CD19, Gr-1, FcεRI, NK1.1, and TER-119) and c-Kit$^+$Sca-1$^+$CD25$^+$CD127$^+$ cells. Lung ILC2s constitutively expressed ICOS as previously reported, and ICOS expression increased after papain treatment (Fig. 1B). We next analyzed the expression of other costimulatory molecules and found that lung ILC2s from PBS-treated mice marginally expressed CD28, programmed cell death-1 (PD-1), its ligand PD-L1 (also known as B7-H1), and receptor activator of NF-κB ligand (RANKL) (Supplemental Fig. S1). CD28 expression slightly decreased and PD-1 expression increased after papain inhalation (Supplemental Fig. S1). Glucocorticoid-induced TNFR family-related gene (GITR), herpes virus entry mediator (HVEM), and Fas were clearly expressed on resting lung ILC2s, and Fas expression increased with papain injection (Fig. 1C). Although these three molecules were expressed in large populations of Lineage$^+$ cells, ICOS was only expressed in a few Lineage$^+$ cells (Fig. 1D). ICOS was expressed on a portion of CD3$^+$ T cells (Fig. 1E). Thus, the ICOS-expressing cells in the lungs included ILC2s and some T cells. Therefore, we focused on the role of ICOS in lung ILC2 activity because of its substantial and relative limited expression.

3.2. ICOS/B7RP-1 interaction increases cytokine production by ILC2s

To reveal whether ICOS/B7RP-1 interaction leads to ILC2 activation, ILC2s were purified from lungs of naïve C57BL/6 mice and cocultured with B7RP-1-transfected P815 (B7RP-1/P815) cells or P815 parental cells. IL-5 and IL-13 production by ILC2s was not observed from coculture with B7RP-1/P815 or P815 cells (Fig. 2), which indicates that ICOS signaling alone could not elicit cytokine production by ILC2s. We next investigated whether ICOS enhances cytokine production by ILC2s in the presence of stimulants. A combination of IL-33 with IL-2, but not IL-33 alone, induces production of cytokines by ILC2s purified from naïve mouse lungs *in vitro* [6]. As reported, the high amounts of IL-5 and IL-13 were