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Rehmannia glutinosa polysaccharide functions as a mucosal adjuvant to induce dendritic cell activation in mediastinal lymph node

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ABSTRACT

In our previous study, we showed that *Rehmannia glutinosa* polysaccharide (RGP) treatment induced activation of dendritic cells (DCs) in human and mouse subjects. In this study, we evaluated the effect of RGP as a mucosal adjuvant for inducting activation of DCs in the mediastinal lymph node (mLN) in the mouse. The C57BL/6 mice were intranasally (*i.n.*) treated with RGP and activation of DC in the mLN was analyzed. The treatment with RGP induced a substantial increase in the number of DCs in the mLN due to the up-regulation of C-C motif chemokine receptor 7 (CCR7) in the DCs. Moreover, the expression of co-stimulatory molecules in the mLN DCs and the concentration of pro-inflammatory cytokines in the lung were up-regulated by RGP treatment. Also, RGP treatment induced interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) production in the mLN T cells. The combination treatment of RGP and ovalbumin (OVA) induced OVA-specific TCR transgenic I (OT-I) and OT-II cell proliferation in the mLN. Finally, the combination treatment of RGP and tyrosinase-related protein 2 (TRP2) peptide, a melanoma self-antigen, protected mice from melanoma challenge. Thus, these data demonstrated that RGP can be used as a mucosal adjuvant for inducing activation of immune responses in the lung.

1. Introduction

Most pathogens enter the body through a mucosal surface [1]. For protection against pathogens, which enter through the mucosal surface, the mucosal epithelial barrier is an effective shield [1,2]. However, to induce immune activation, the epithelial barrier is a major obstacle because it inhibits delivery of adjuvants (immune stimulatory molecules) in the antigen-presenting cells (APCs) [2]. The activation of APCs in the mucosal tissues is required to induce vaccine activates for treatment and prevention against infectious diseases and cancer [1,3,4]. Therefore, the ability of adjuvants to promote APC activation is essential for effective treatment of infectious diseases and cancer in mucosal tissues.

Among APCs, dendritic cells (DCs) are the most powerful [5,6]. The pathogen contains molecules that stimulate DCs *via* pattern recognition receptors (PPRs) such as toll-like receptors and scavenger receptors [7–9]. Activated DCs up-regulate the expression of co-stimulatory molecules and produce pro-inflammatory cytokines. Moreover, the DCs present antigens to T cells after migrate lymph node (LN). Maturation of DCs is defined by high levels of co-stimulatory molecules, production of pro-inflammatory cytokines, and presentation of antigen to T cells [7,10,11]. In mice, DCs have two main subsets: $CD8\alpha^+$ and $CD8\alpha^-$ DCs. $CD8\alpha^+$ DCs induce CD8 T cell activation by cross-presentation of antigens, and $CD8\alpha^-$ DCs directly present antigens to CD4 T cells [12–15]. For induction of the therapeutic effect against infectious disease and cancer, the activation of an antigen-specific CD8 T cell, called the cytotoxic T lymphocyte (CTL), is required [4,11,16].

Recent studies have suggested that natural polysaccharides, such as fucoidan, laminarin, ascophyllan, and pullulan, can induce activation of DCs in mice [10,14,17]. We found that *Rehmannia glutinosa* polysaccharide (RGP) also functions as an adjuvant for the promotion of DC activation in humans and mice [7,18]. RGP is made up of arabino-3, 6-galacan type structural units and composed of L-arabinose: D-galactose: L-rhamnose: D-galaturonic acid in the molar ratios of 10:10:1:1 [19,20]. However, RGP was not evaluated to determine whether it can induce DC

Abbreviations: RGP, *Rehmannia glutinosa* polysaccharide; CTL, cytotoxic T lymphocyte; CFSE, carboxyfluorescein diacetate succinimidyl ester; DC, dendritic cell; CCR7, C-C motif chemokine receptor 7; IL, interleukin; IFN, interferon; LPS, lipopolysaccharide; MHC, major histocompatibility complex; Th, T helper; TNF, tumor necrosis factor; Ovalbumin, (OVA); Ovalbumin-specific TCR transgenic, OT.

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activation in mediastinal LN (mLN) by intranasal (*i.n.*) injection. Since the intravenous (*i.v.*) injection of RGP-induces maturation of DCs in mice *in vivo*, we hypothesized that *i.n.* injection of RGP may promote activation of DCs in mLN and antigen-specific T cell immune responses; the current study was undertaken to test this hypothesis.

2. Results

2.1. Activation of dendritic cells in mediastinal lymph node was induced by intranasal injection of RGP

Since *i.v.* injection of RGP promotes spleen DC activation [7], we next examined the mLN DC activation by *i.n.* injection of RGP. C57BL/6 mice were *i.n.* injected with 50 mg/Kg RGP. Lipopolysaccharide (LPS; 1 mg/Kg) was also used in the mice as a positive control. Twenty-four hours after injection, the mLN was harvested, and a number of DCs were analyzed. The mLN DCs were defined as lineage$^-$CD11c$^+$ live leukocytes, and the cells were further divided into CD8α$^+$ and CD8α$^-$ DCs (Fig. 1A). The *i.n.* treatment of RGP induced significant increases in the lineage$^-$ CD11c$^+$ cells in the mLN, which was almost similar to LPS treatment (Fig. 1B). Moreover, the RGP treatment induced up-regulation of CCR7 expression on the surface of DCs in the mLN, which indicates the DCs migrated to the LN due to the stimulation of RGP (Fig. 1C).

Next, we measured co-stimulatory molecule expression and pro-inflammatory cytokine production. The mLN DCs were divided into CD8α$^+$ and CD8α$^-$ DCs (Fig. 1A) and further analyzed for co-stimulatory molecule expression. It was found that the *i.n.* treatment of RGP in mice promoted substantial up-regulation of CD40, CD80, CD86, and major histocompatibility complex (MHC) class I and II expression in both CD8α$^+$ and CD8α$^-$ DCs in mLN (Fig. 2A). Moreover, the concentration of interleukin-6 (IL-6), IL-12p40 and tumor necrosis factor-α (TNF-α) levels were markedly increased by RGP treatment in bronchoalveolar lavage fluid (Fig. 2B). Thus, these data suggested that *i.n.* administration of RGP can induce activation of DCs in mLN.

2.2. RGP promotes antigen-specific T cell activation in mLN

We next examined whether RGP promotes T cell activation. C57BL/6 mice were *i.n.* injected with 50 mg/Kg of RGP and 1 mg/Kg LPS; three days later, the mice were injected with the same amount of RGP and LPS again. The intracellular levels of IFN-α increased dramatically in the RGP-treated CD4 and CD8 T cells in mLN (Fig. 3A and B). Moreover, the TNF-α levels were also substantially up-regulated by RGP in CD8 T cells, whereas CD4 T cells did not produce TNF-α by RGP (Fig. 3A and B). Thus, these data suggested that *i.n.* administration of RGP can induce Th1 and Tc1 immune responses.

Our finding that *i.n.* treatment of RGP induced activation of DCs and T cells in mLN prompted us to examine whether *i.n.* injection of RGP can induce antigen-specific immune responses in mLN. To evaluate antigen-specific immune activation, we transferred OT-I and OT-II cells into CD45.1 congenic mice. Twenty-four hours after cell transfer, the mice were *i.n.* treated with phosphate-buffered saline (PBS), OVA, RGP, and a combination of RGP and OVA. The proliferation of OT-I and OT-II cells in mLN were remarkably increased by the combination of RGP and OVA, while PBS, OVA, and RGP alone treatment did not induce proliferation of the cells (Fig. 3C). The increased rates of T cell proliferation were almost similar to those produced by LPS treatment. Therefore, these data suggested that the combination treatment of RGP and antigen by *i.n.* promoted antigen-specific immune activation in mLN.

2.3. The combination treatment of RGP and antigen inhibits cancer growth in the lung

Since RGP and OVA treatment promoted OVA-specific immune activation in mLN, we next examined the anti-cancer effect with a

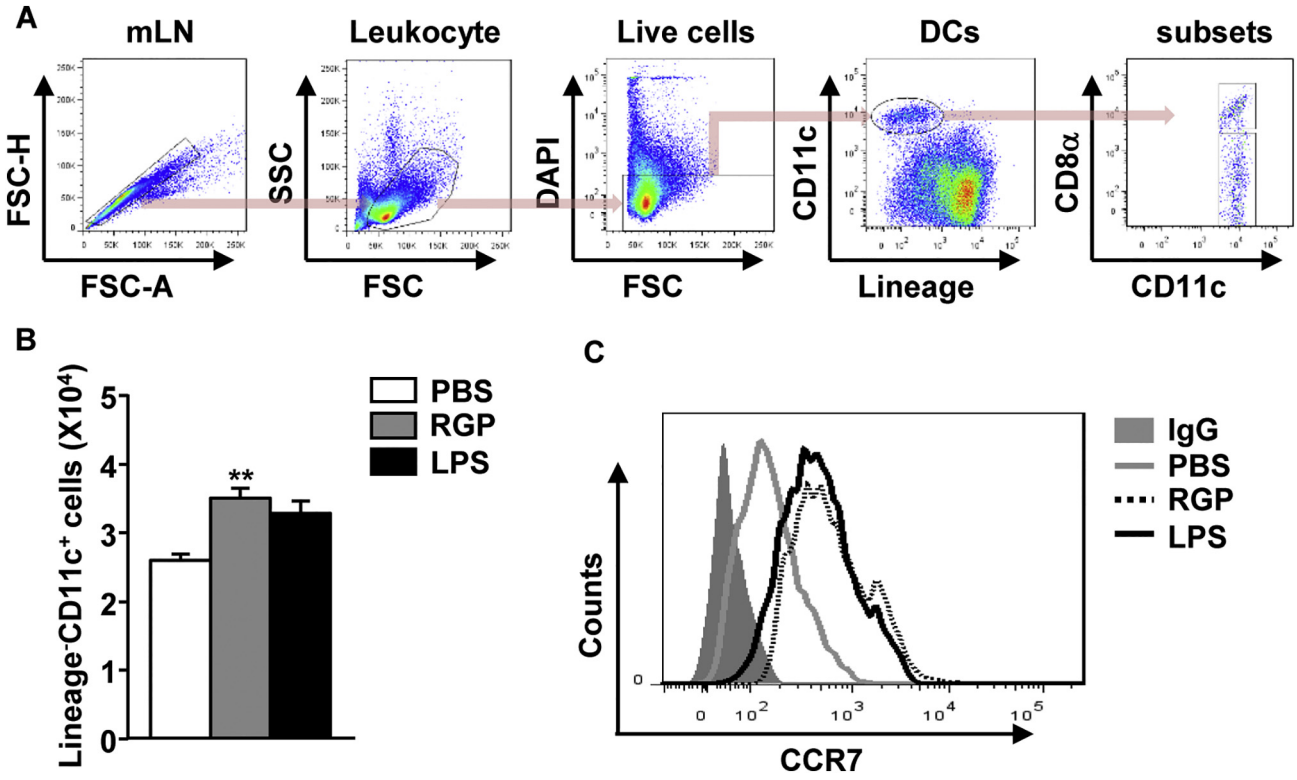

Fig. 1. *RGP* induced migration of dendritic cells in mediastinal lymph node. C57BL/6 mice were injected intranasally with PBS, 50 mg/Kg of RGP and 1 mg/Kg of LPS. Twenty-four hours after injection, mLN was harvested and analyzed. (A) The population of DCs in mLN was determined as lineage$^-$CD11c$^+$ cells in live leukocytes. Lineage markers included CD3, Thy1.1, B220, Gr-1, CD49b, and TER-119. The CD11c$^+$lineage$^-$ DCs were further divide to CD8α$^+$ and CD8α$^-$ DCs. (B) Mean number of lineage$^-$CD11c$^+$ cells in the mLN. (C) Expression levels of CCR7 are shown. All data are representative of or the average of analyses of 6 independent samples (2 mice per experiment, total 3 independent experiments). $^{**}p < 0.01$.