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# Respiratory macrophages and dendritic cells mediate respiratory syncytial virus-induced IL-33 production in TLR3- or TLR7-dependent manner

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ABSTRACT

Respiratory syncytial virus (RSV) infection can increase the production of IL-33 in lungs of mice. However, little is known about cellular source of IL-33, particularly the types of IL-33-producing cells in innate immune cells during RSV infection. In this study, by using BALB/c mice that were infected intranasally with RSV, it became clear that RSV infection can enhance not only the number of IL-33$^{+}$-alveolar macrophages (AMs) and dendritic cells (DCs), but also the expression of IL-33 mRNA in these cells, suggesting that innate immune cells participate in the production of IL-33. Indeed, in vitro experiments by using murine cell lines found that RSV infection results in more expression of IL-33 mRNA in AMs and DCs, further confirming that these cell types may be an important source of IL-33 during RSV infection. It should be noted that the expression of mRNA for TLR3 and TLR7 was up-regulated in pulmonary AMs during RSV infection. Blockade of TLRs by TLR3 or TLR7 antagonist significantly reduces the levels of IL-33 mRNA in AMs and DCs, suggesting that RSV-induced IL-33 production might be TLRs-dependent manner. Although the expression of TLRs mRNA in pulmonary interstitial macrophages (IMs) was enhanced after RSV infection, stimulation with agonists or inactivated RSV cannot alter the expression of IL-33 mRNA in IMs, suggesting that pulmonary IMs may not be a source of IL-33 during RSV infection. Thus, these results demonstrate that during RSV infection, respiratory macrophages and dendritic cells mediate the production of IL-33 in a TLR-dependent manner.



## 1. Introduction

Asthma is an immune-mediated disease characterized by the infiltration of eosinophils and the production of type 2 cytokines by T lymphocytes in response to certain antigens [1–3]. As a most common cause of respiratory-tract infections, respiratory syncytial virus (RSV) infection has been linked to asthma and has been shown to elicit asthma exacerbation [4,5]. Although the precise mechanisms for the development of asthma and allergy by RSV infection are not clear, a relative predominance of Th2 over Th1 cytokine has been demonstrated to be important [6].

IL-33 is a novel member of the IL-1 cytokine family and a ligand for the orphan IL-1 family receptor ST2 [7]. Unlike the other IL-1 family members, IL-33 primarily induces Th2-immune responses in a number of immune cell types [8]. It is clear that IL-33 can active murine dendritic cells directly driving polarization of naïve T cells towards a Th2 phenotype [9]. Moreover, IL-33 can act directly on Th2 cells to augment production of Th2 cytokines, such as IL-13 and IL-5 [10,11], and it can also act as a chemo-attractant for Th2 cells [12]. In fact, treatment of mice with anti-IL-33 mAb can inhibit the activity of allergen-specific Th2 cells, reduce pulmonary eosinophil infiltration and down-regulate airway hyper-responsiveness [13,14], further confirming that IL-33 plays a critical role in antigen-induced Th2-related airway diseases.

IL-33 is present mainly in the nuclei of epithelial cells [15] and endothelial cells [15,16] and released as an alarmin especially when considering the event of tissue damage [8]. Recently, increasing evidence suggests that innate immune cells might be also an important cellular source of IL-33, particularly during respiratory virus infection. Indeed, infection of alveolar macrophages and bone marrow-derived dendritic cells with influenza A virus results in more IL-33 production, in contrast, infection of mouse lung epithelial cell line with the same influenza A virus did not alter the expression of IL-33 mRNA [17], suggesting that under a certain condition, innate immune cells might be a major source of IL-33.

It has been reported that intranasal infection with RSV can increase the production of IL-33 in the lungs of BALB/c mice [18]. However, little is known about cellular source of pulmonary IL-33, particularly the types of innate immune cells for IL-33 production during RSV infection. Thus, in the present study, we focus on innate immune cells, especially

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alveolar macrophages, dendritic cells and interstitial macrophages in the lungs of RSV-infected mice, to investigate the cellular source of IL-33 and the mechanisms for IL-33 production.

## 2. Materials and methods

### 2.1. Virus

Human respiratory syncytial virus type A2 (RSV A2) was supplied kindly by Dr. B. Wang, Center for Disease Control and Prevention, Shenyang, China. The virus was propagated in monolayer of HEp-2 cells (ATCC). At the time point of the maximum cytopathic effect, cells were disrupted by sonication and the virus was purified by a sucrose gradient centrifugation [19]. Virus titer was expressed as a 50% tissue culture infectious dose (TCID50), calculated using the method of Reed and Muench [20]. To inactivate the virus, the titrated virus was irradiated with ultraviolet (UV) light for 15 min on ice [21].

### 2.2. Mice

Specific-pathogen-free, 6-week-old female BALB/c mice were purchased from Shanghai Laboratory Animal Center. Mice were anesthetized mildly by intraperitoneal administration of chloral hydrate (0.229 mg/g body weight) and infected intranasally with RSV in 20 μl of sterile phosphate-buffered saline (PBS) containing $2 \times 10^6$ TCID50 per mouse. As a control group (expressed as day 0 in figures), mice were inoculated intranasally with sterile PBS. For blocking the function of IL-33, some mice were treated intranasally with 5 μg of anti-IL-33 (R&D systems) or isotype-matched control antibody (R&D systems) at 1 h before and daily after infection [22]. At intervals, samples were collected. This work was approved by the Institutional Animal Care and Use Committee of China Medical University, China.

### 2.3. Histopathological analysis

Lungs were lavaged through an intratracheal tube with 1 ml of PBS. After centrifugation, the pellet was then cytospun on to glass slides and stained with Wright–Giemsa. Two hundred cells were counted on each slide and the proportions of different leukocyte subtypes were calculated. Whole lungs were then removed and fixed in 4% paraformaldehyde. The lungs were embedded in paraffin and lung tissue sections (5 μm thick) were stained with hematoxylin and eosin (H&E) for analysis of peribronchial cellular infiltration.

### 2.4. Preparation of single cell suspensions from the lung

Mice were anesthetized and the lung was flushed in situ with 20 ml of PBS via cannulation of the heart to remove intravascular blood pool. Lungs were minced and incubated at 37 °C for 1 h on a rocker with 200 μg/ml collagenase D and 40 μg/ml DNase I (Roche Molecular Biochemicals) in 10 ml of RPMI 1640 medium. Single cell suspensions from the digested lung were collected through density-gradient centrifugation with lymphocyte-separation solution and resuspended in RPMI 1640 medium.

### 2.5. Flow cytometry

Single-cell suspensions from lung parenchyma were blocked firstly with anti-mouse CD16/32 (BD Biosciences) and then, incubated on ice for 20 min with either specific mAbs or isotype-matched control Igs. Monoclonal Abs specific to F4/80, CD11c, CD45 were purchased from BioLegend. 100,000 stained lung cells were analyzed by flow cytometry. Depending on references, AMs, DCs and IMs in the lung parenchyma were identified as $CD45^+CD11c^+F4/80^+$, $CD45^+CD11c^+F4/80^-$, $CD45^+CD11c^-F4/80^+$, respectively [17,23].

### 2.6. Ex vivo intracellular cytokine assay

Cells were surface stained with anti-CD45, anti-F4/80 and anti-CD11c Abs for 30 min at 4 °C, followed by fixation and permeabilization with Cytofix/Cytoperm (BD Biosciences). Intracellular cytokine staining for IL-33 (PE-conjugated anti-IL-33 mAbs was purchased from R&D systems) was assayed in accordance with the manufacturer's instruction.

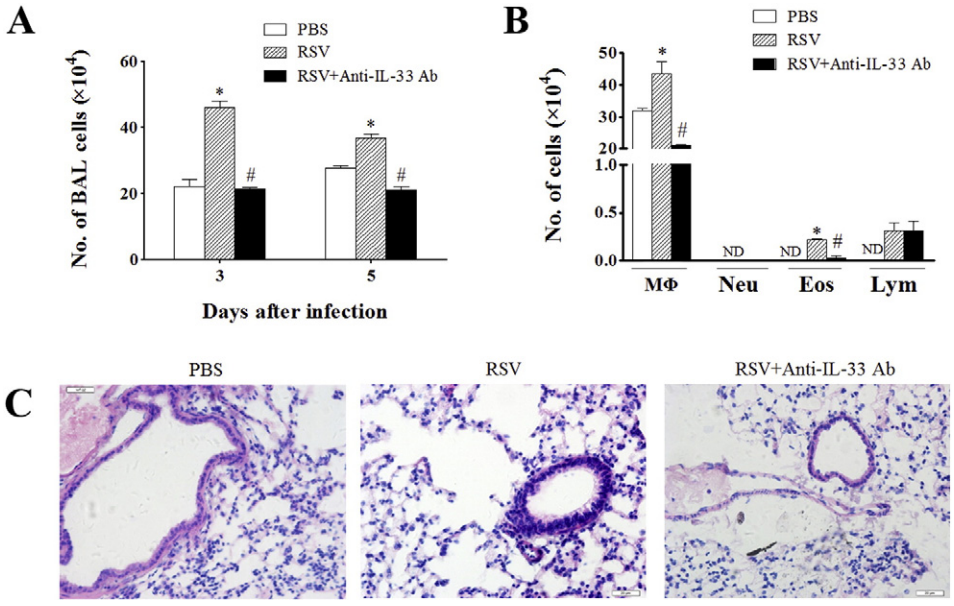


**Fig. 1.** Treatment with anti-IL-33 antibody diminishes RSV-induced airway inflammation. BALB/c mice were infected intranasally with RSV at an inoculum dose of $2 \times 10^6$ TCID50 per mouse. 5 μg of anti-IL-33 antibody was inoculated intranasally into the mice at 1 h before and daily after infection. The number of total BAL cells were detected at the indicated time points (A). The proportions of different leukocyte subtypes in BAL fluids were calculated on day 3 after infection (B). Lung pathologic lesions were analyzed on day 3 after infection (C). Data are representative of three independent experiments with five mice for each group. *Significant difference ($P < 0.01$ by ANOVA), compared with PBS-inoculated mice. #Significant difference ($P < 0.01$ by ANOVA), compared with corresponding non-anti-IL-33 mAb-treated mice. ND, not detectable.