Video Article

Analysis of Pulmonary Dendritic Cell Maturation and Migration during Allergic Airway Inflammation

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Abstract

Dendritic cells (DCs) are the key players involved in initiation of adaptive immune response by activating antigen-specific T cells. DCs are present in peripheral tissues in steady state; however in response to antigen stimulation, DCs take up the antigen and rapidly migrate to the draining lymph nodes where they initiate T cell response against the antigen^{1,2}. Additionally, DCs also play a key role in initiating autoimmune as well as allergic immune response³.

DCs play an essential role in both initiation of immune response and induction of tolerance in the setting of lung environment⁴. Lung environment is largely tolerogenic, owing to the exposure to vast array of environmental antigens⁵. However, in some individuals there is a break in tolerance, which leads to induction of allergy and asthma. In this study, we describe a strategy, which can be used to monitor airway DC maturation and migration in response to the antigen used for sensitization. The measurement of airway DC maturation and migration allows for assessment of the kinetics of immune response during airway allergic inflammation and also assists in understanding the magnitude of the subsequent immune response along with the underlying mechanisms.

Our strategy is based on the use of ovalbumin as a sensitizing agent. Ovalbumin-induced allergic asthma is a widely used model to reproduce the airway eosinophilia, pulmonary inflammation and elevated IgE levels found during asthma^{6,7}. After sensitization, mice are challenged by intranasal delivery of FITC labeled ovalbumin, which allows for specific labeling of airway DCs which uptake ovalbumin. Next, using several DC specific markers, we can assess the maturation of these DCs and can also assess their migration to the draining lymph nodes by employing flow cytometry.

Video Link

The video component of this article can be found at <http://www.jove.com/video/4014/>

Protocol

1. Sensitization of Mice with Ovalbumin

1. Prepare a solution of OVA (grade V; Sigma, MO) in sterile PBS at a concentration of 1 mg/ml (solution can be stored at -80 °C).
2. In order to prepare OVA-Alum mixture, take Alum in a tube and add OVA solution in a dropwise fashion while vortexing the tube at a ratio of 1:1. Stir the mixture for 30 minutes and use right away after mixing.
3. Using a 1 ml syringe, inject 0.2 ml of the mixture into mouse peritoneal cavity and repeat the injection again after 2 weeks.

2. Intranasal Challenge of Mice with FITC Labeled Ovalbumin

1. 7 days after the second injection of OVA-Alum, mice are ready to be challenged intranasally with OVA-FITC.
2. Prepare a solution of OVA-FITC (Sigma) using sterile PBS at a concentration of 1 mg/ml and store in aliquots at -80 °C.
3. Place a sterile gauze at the bottom of a 50 ml falcon tube and in a chemical hood, add 5 ml of Aerrane onto the gauze. In order to anesthetize mice, direct the animal into the tube for approximately 5-10 seconds.
4. Hold the anesthetized animal in an upright position and using sterile pipette tips, pipette 100 µl of the OVA-FITC solution onto the nares of mice in a drop-wise fashion.
5. Repeat intranasal challenge with OVA-FITC for the next two days.

3. Preparation of Single-cell Suspension from the Lungs and the Draining Lymph Nodes

1. Sacrifice the mice using intraperitoneal injection of Euthanyl.

2. In order to reduce macrophage contamination, which can complicate DC analysis, lung lavage is performed. To lavage the lungs, mouse tracheas are briefly exposed and cannulated with a catheter and a syringe; using ice-cold PBS with 5 mM EDTA, the lower respiratory tract is rinsed 3 times to remove cells from the alveolar spaces.
3. Perfuse the lungs with 10 ml of PBS containing 10 U/ml heparin via the right ventricle of the heart, to remove blood cells from the lung vasculature. Perform the perfusions till the lungs turn white, indicative of removal of most of the blood cells. This is especially important to remove contaminating cells from the peripheral blood.
4. Dissect the lungs out and remove mediastinal lymph nodes (MLN) and place the lymph nodes in a separate dish. (Try to minimize exposure of tissues to light to prevent quenching of FITC fluorescence.)
5. Place the lungs on a Petri dish and mince using scissors. Next digest the lungs for 25 minutes at 37 °C using 250 U/ml Collagenase D (Roche) and add EDTA (10 mM final concentration) for the next 5 minutes to stop collagenase activity.
6. Pass the fragments of digested lungs through a 100 μ m cell strainer and perform hypotonic lysis to remove erythrocytes. The single cell suspension is ready for further analysis of DCs.
7. In order to prepare single cell suspension from the MLN, tease the MLN using fine needles and digest with collagenase as described above followed by straining through a cell strainer.

4. Staining for DC Markers to Assess Maturation/migration

1. In order to identify DCs in the lungs, staining is performed for CD11c and CD11b. Furthermore to assess for maturation, staining is performed for CD86 and CD80, which are upregulated as DCs undergo maturation. CD11c⁺CD11b⁺OVA-FITC⁺ cells in the MLN are identified as pulmonary DCs migrating from the lungs to the MLN in response to OVA airway challenge.
2. Suspend cells at a concentration of 10×10^6 cells/ml in FACS buffer (PBS with 1% FBS and 1mM EDTA) and aliquot 150 μ l of cell suspension / tube for FACS staining. For identification of lung DCs, following stains are needed: Unstained control (Lung cells from mice not exposed to OVA-FITC), OVA-FITC control, CD11c single control, CD11b single control, CD11b⁺CD11c⁺ double control, CD11b⁺ OVA-FITC⁺ double control, CD11c⁺ OVA-FITC⁺ double control, MHC II single control, CD86 single control, CD80 single control and samples stained for OVA-FITC⁺CD11c⁺CD11b⁺MHCII⁺, OVA-FITC⁺CD11c⁺CD11b⁺CD86⁺ and OVA-FITC⁺CD11c⁺CD11b⁺CD80⁺.
3. To assess DC migration, perform absolute cell counts of the single cell suspension from MLN and subsequently prepare the following tubes: Unstained control (MLN cells from mice not exposed to OVA-FITC), OVA-FITC control, CD11c⁺ single control, CD11b⁺ double control, CD11b⁺ OVA-FITC⁺ double control, CD11c⁺OVA-FITC⁺ double control and CD11c⁺CD11b⁺OVA-FITC⁺ samples.

5. Representative Results

The time points required for intraperitoneal sensitization to induce airway allergic inflammation is important and should be carried out as depicted in **Figure 1**. Following intraperitoneal sensitization and airway OVA challenge, to confirm induction of airway allergic inflammation, some mice can be sacrificed and histological analyses can be carried out on the lung sections as shown in **Figure 2**. Presence of inflammatory cells can be confirmed by Hematoxylin & Eosin stain (**Figure 2A**) and presence of mucus production can be assessed by Periodic-acid-Schiff stain (**Figure 2B**). Altogether this will confirm induction of airway allergic inflammation following OVA challenge of OVA-sensitized mice⁸. In contrast, lung sections from Saline treated mice are expected to be free of any inflammation along with absence of any mucus production. Moreover, following OVA challenge, pulmonary DCs undergo maturation and subsequent migration to the draining lymph nodes. Analysis of CD11c⁺ cells in the mediastinal lymph nodes (MLN) from mice sensitized and challenged with OVA is expected to show a higher proportion of CD11c⁺ cells compared to saline-treated mice (**Figure 3A**). Moreover, analysis of absolute cell count of CD11c⁺CD11b⁺OVA-FITC⁺ cells in the MLN of OVA-sensitized and challenged mice, is expected to show a significantly higher (i.e. several fold higher) count than the counterparts from saline treated mice (**Figure 3B**).

Figure 1. Experimental protocol for induction of ovalbumin (OVA) induced allergic airway inflammation in mice along with airway challenge with FITC labeled ovalbumin (OVA-FITC).

Figure 2. OVA sensitization followed by OVA airway challenge leads to induction of airway allergic inflammation as identified by Hematoxylin & Eosin staining of lung sections shown in (A) and also leads to mucus production in the airways as identified by Periodic Acid Schiff staining of lung sections as shown in (B).

Figure 3. OVA-FITC challenge induces DC migration from the lungs to the mediastinal lymph nodes (MLN). (A) Flow cytometry plots depicting proportions of CD11c⁺ cells in the MLN of control or OVA-sensitized mice. (B) Absolute counts of CD11c⁺CD11b⁺OVA-FITC⁺ cells in the MLN of saline or OVA-sensitized mice. * $p < 0.05$. [Click here to view larger figure.](#)

Discussion

The method presented here offers a flow cytometry based approach for analyzing pulmonary DCs, based on delivery of OVA-FITC for airway challenge. This allows for selective monitoring of pulmonary DCs, which take up OVA-FITC and therefore the DC populations, which are monitored are effectively the ones that are participating in the airway immune response during the course of OVA-induced allergic airway inflammation. In control mice, in the absence of allergic airway inflammation, the numbers of migratory DCs (OVA-FITC⁺ DCs) identified in the MLN are indicative of basal rates of DC migration, which accelerates in response to allergic airway inflammation resulting in significant increase in the absolute count of OVA-FITC⁺ DCs in the MLN. The described strategy, offers advantage over traditional immunohistochemical/immunostaining based approaches because analysis can be carried out in shorter time span and the efficiency/sensitivity is much higher. Therefore it is equally important to ensure that proper controls as described in the methods are employed during flow cytometry analysis. Another parameter that can affect the results is the preparation of single cell suspension from the lung. Hence care must be taken to prevent significant exposure of the tissue to light for it may affect the fluorescence of OVA-FITC and care must also be taken during enzymatic digestion of the lung for over digestion may lead to cleavage of surface markers on cells, which will affect downstream analysis. Alveolar macrophages and DCs share similar sets of surface markers, which further complicates the analysis of pulmonary DC populations⁹. Therefore it is crucial to lavage the lungs to remove alveolar macrophages which can interfere with DC analysis.

In addition to the analysis of pulmonary DC migration during allergic airway inflammation, the described methodology can be potentially modified to assess immune response to other antigens, whereby specific antigens can be labeled with a fluorescent dye and delivered intranasally. Subsequently similar analyses as described above can be carried out to assess pulmonary DC response. In cases where antigen cannot be labeled, few hours prior to antigen delivery, FITC-dextran or CFSE can be delivered to mice. Subsequently, after antigen delivery, analysis can be carried out to monitor migration of FITC-dextran or CFSE labeled DCs to the MLN, which will indicate the kinetics of pulmonary DC migration following inoculation with the specific antigen^{10,11}. In our previous work, we have employed this strategy to assess maturation/migration of pulmonary DCs in response to delivery of adenoviral gene therapy vectors¹⁰. Since DCs are phagocytic, intranasal delivery of FITC-Dextran results in rapid uptake by pulmonary DCs, which can then be monitored by flow cytometry. In cases, where monitoring of pulmonary plasmacytoid DCs is desired, CFSE should be employed since plasmacytoid DCs do not uptake FITC-Dextran as efficiently as other pulmonary DC subsets¹⁰.

Altogether, the described strategy offers advantages over other traditional approaches for it offers a quantitative analysis of pulmonary DC maturation/migration during the course of airway inflammation.

Disclosures

No conflicts of interest declared.

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