



Technical note

An efficient single prime protocol for the induction of antigen-induced airways inflammation

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ARTICLE INFO

Article history:

Received 15 May 2013

Received in revised form 19 June 2013

Accepted 19 June 2013

Available online 4 July 2013

Keywords:

Allergic airways inflammation

Single prime

Antigen sensitization

Ovalbumin

Th2 response

ABSTRACT

There are a number of mouse models of allergic airway inflammation used to delineate various aspects of asthma. One of the hurdles with using mice is their natural resistance to developing a Th2 allergic response. This is often overcome by double priming with the allergen and an adjuvant. Here we report on an efficient 11 day, single antigen/alum priming protocol that is sufficient to sensitise mice for the development of Th2-mediated inflammation in the lung following antigen challenge.

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1. Introduction

There are a number of methods for inducing allergic airway inflammation in mice as a model of asthma in humans (Nials and Uddin, 2008; Finkelman and Wills-Karp, 2008; Mathrani et al., 2007). Modelling asthma in mice to reflect the human disease is difficult due to a number of differences between mice and humans, including reduced susceptibility to allergen challenge (Nials and Uddin, 2008). This lack of susceptibility can be circumvented by priming the mice with the allergen and an adjuvant at a remote site prior to commencing allergen challenge.

One of the common model allergens used in research is the egg protein ovalbumin (OVA) (Kumar et al., 2008; Park et al., 2007). Currently, the gold standard practice for the OVA/alum model of lung inflammation is to prime intraperitoneally (i.p.) twice, one to two weeks apart, prior to intranasal (i.n.) or aerosol challenge with OVA (Pichavant et al., 2007). However, there is evidence that a single injection of OVA in alum potently induces antigen-specific IgE indicating that a single prime may be

sufficient for antigen sensitization to raise a Th2 response (Beck and Spiegelberg, 1989). Here, we report that OVA-mediated allergic airways inflammation can be induced in C57BL/6J mice using a single priming dose of OVA, significantly shortening the experimental time required to undertake Th2-mediated studies of antigen-specific airways inflammation.

2. Methods

2.1. Mice

C57BL/6J male mice were maintained in a conventional animal facility at the Malaghan Institute of Medical Research, Wellington, New Zealand. All experimental procedures were approved by the Victoria University of Wellington Animal Ethics Committee.

2.2. Materials

Ovalbumin (OVA), 4% formalin, Tween20, foetal bovine serum (FBS) and ketamine/xylazine were from Sigma (Auckland, NZ). Alum was from Serya (Heidelberg, Germany). Antibodies against mouse CD3e, CD8a, CD4, CD11b, CD11c, and Gr-1 were from Biolegend (San Diego, CA). Anti-mouse SiglecF, MHCII

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and CD45 and TMB reagent were from BD Biosciences (San Jose, CA). ELISA plates were from Greiner Bio-One (Frickenhause, Germany) and streptavidin-HRP was from R and D Systems (Minneapolis, MN). Anti-mouse IgE (clone 6HD5) and biotinylated anti-mouse IgE (clone R1E4) were produced “in-house”. Unless otherwise stated, all buffers were from Life Technologies (Auckland, NZ).

2.3. Single prime OVA-induced airway inflammation model

Allergic airway disease was induced using OVA as the model allergen. Mice were primed i.p. with 100 µg OVA in 200 µL alum adjuvant on day 0. On day 7 mice were anaesthetised with 1 µL/g ketamine/xylazine and challenged i.n. with 100 µg OVA in PBS. On day 11, mice were sacrificed (ketamine/xylazine overdose) and bronchial-alveolar lavage fluid (BALF), serum, mediastinal lymph nodes and lung tissue were collected.

2.4. Cell and tissue analysis

Cellular counts were performed on the BALF and lymph nodes to determine total cell infiltrate. Cells were stained with fluorophore-conjugated antibodies for key surface markers to identify monocytes/macrophages (CD45 +/CD11b +/Cd11c +/MHCIIlow), eosinophils (CD45 +/CD11b +/siglecF +), T cells (CD45 +/CD3e +/CD8a + or CD4 +), neutrophils (CD45 +/CD11b +/Gr-1hi) and antigen presenting cells/dendritic cells (CD45 +/CD11c +/MHCIIhi) by flow cytometry. Lung tissue was fixed in 4% formalin before being sectioned and stained

with haematoxylin and eosin (H&E) or Alcian-blue Periodic acid-Schiff (AB-PAS) stains (Dept of Pathology, Wellington School of Medicine, University of Otago, Wellington, NZ). Total IgE in the serum was quantified by ELISA.

2.5. Statistical analysis

All statistical analysis was performed using GraphPad Prism V.5. software. The data were analysed using an unpaired student t-test.

3. Results and discussion

To confirm the induction of lung inflammation by OVA challenge after single OVA/alum priming, lung tissue was collected four days after intranasal OVA challenge and stained with H&E. This time point is in line with other models that show peak eosinophilia alongside development of Th2 responses and airway remodelling (Nials and Uddin, 2008).

As shown in Fig. 1A and B OVA challenge induced considerable cellular infiltration and airway thickening in the lung compared to naïve mice. AB-PAS staining of lung tissue also identified a significant number of mucous positive goblet cells within the bronchioles of OVA-challenged mice consistent with the induction of Th2 immune responses in the lung.

Along with histological evidence of OVA-induced lung inflammation, analysis of the infiltrating cells in the BALF by flow cytometry confirmed a significant increase in cellular infiltration into the lung of OVA challenged mice (Fig. 2A). Breakdown analysis of the cellular profile showed significant

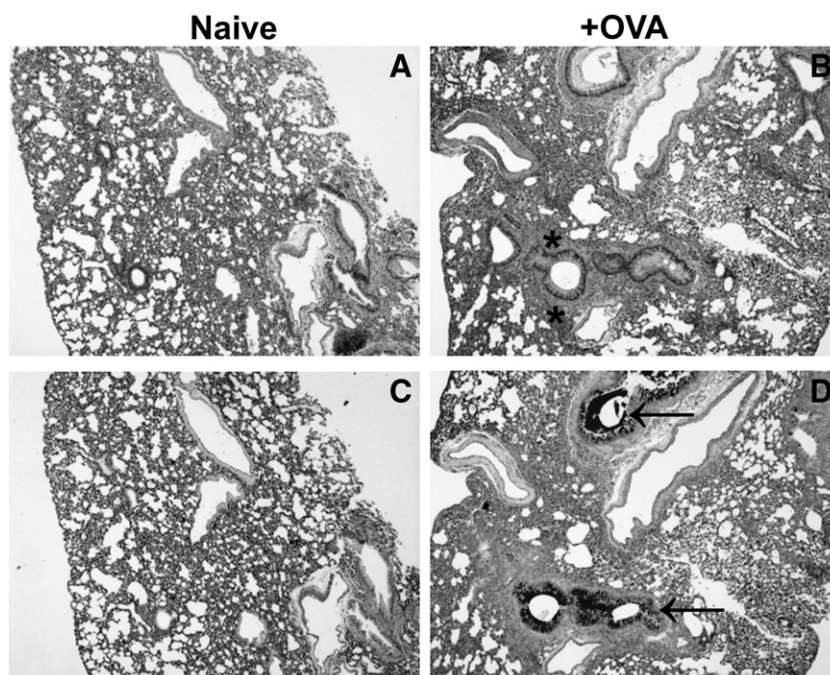


Fig. 1. Histological analysis of lung tissue following single OVA prime and challenge. Mice were primed i.p. with OVA/alum on day 0 then challenged i.n. with OVA on day 7. On day 11 mice were sacrificed and lung tissue sectioned and stained with H&E (A and B) and AB-PAS (C and D) for naïve and OVA challenged mice. H&E staining shows cellular infiltration as indicated by asterisks. The arrows indicate mucous producing goblet in dark black cells by AB-PAS staining. Representative data from 3 separate experiments.

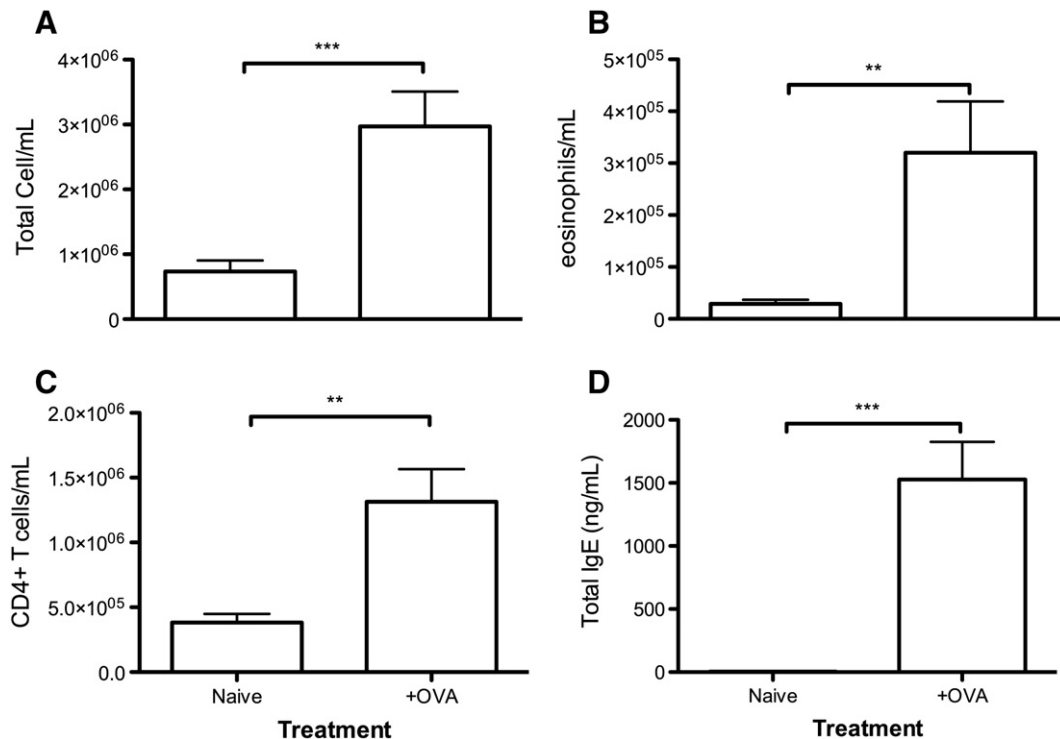


Fig. 2. Cellular and IgE quantification following single OVA prime and challenge. Mice were primed i.p. with OVA/alum on day 0 then challenged i.n. with OVA on day 7. On day 11 mice were sacrificed. To assess the effectiveness of the model BALF was collected and (A) total cell, and (B) eosinophils numbers enumerated. Mediastinal lymph nodes were also harvested and (C) CD4+ T cell numbers were quantified and (D) serum IgE was detected by ELISA. ** $P < 0.01$, *** $P < 0.001$ compared to naïve (unpaired student t-test). Combined data from 3 separate experiments ($n = 15$ per group).

eosinophilia in the BALF (Fig. 2B), consistent with Th2-induced immune responses. Further evidence of this Th2 phenotype could be seen in the accumulation of CD4+ T cells in the lung draining mediastinal lymph node (Fig. 2C) and the significant increase in serum IgE levels (Fig. 2D). No significant increases in the number of neutrophils or APC/DCs were observed (data not shown).

How this shortened priming protocol translates into different mouse strains e.g. Th2 biased Balbc mice is warranted to determine if single priming can be applied into other commonly used models. Extending the analyses to investigate the impact of priming alone on the Th2/eosinophil response in the lung compared to naïve and OVA challenged mice will also be important to fully profile this shortened prime/challenge model.

There are numerous variations in the mouse model of allergic airway inflammation as researchers have attempted to reproduce various aspects of human asthmatic disease (Kips et al., 2003; Nials and Uddin, 2008). These variations include differences in mouse strains, allergen type, method and frequency of delivery (Nials and Uddin, 2008). A common theme in these models is the use of multiple priming events that significantly add to the length of time it takes to perform the experiments. Here we describe a single prime model for inducing allergic airway inflammation that has the same basic characteristics as models using multiple priming events (Last et al., 2004; Kumar et al., 2008). Furthermore, the protocol described here aligns with recent clinical data suggesting that

allergic airways inflammation can occur with minimal priming events (Depner et al., 2013).

4. Conclusions

A single priming event using OVA/alum is sufficient to sensitise C57BL/6J mice for a robust Th2 response in the lung following i.n. challenge with OVA protein. This protocol represents a significant reduction in experimental time compared to the classical OVA double prime/challenge model of Th-2 mediated lung inflammation.

Conflict of interest

The authors declare no conflict of interest.

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