NKT cells and CD8+ T cells are dispensable for T cell dependent allergic airway inflammation

CURRENT STATUS: POSTED

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DOI:
10.1038/nprot.2007.32

SUBJECT AREAS
Biological techniques *Chemical biology*

KEYWORDS
CD4+ T cells, CD8+ T cells, NKT cells, airway inflammation, asthma

Introduction

Allergic airway inflammation (AAI) features airway and peribronchial accumulation of eosinophils and lymphocytes, which infiltrate in response to the cytokines IL-4, IL-5, and IL-13. While type 2 helper T (Th2) cells are clearly important for the development of AAI, it has been reported that natural killer T (NKT) cells and activated memory CD8+ T cells play essential effector roles in AAI. These results call into question the current paradigm that CD4+ helper T cells are the main players in allergic asthma.

Reagents

CIITA and beta2-microglobulin knock out mice in C57BL/6 ($H2^b$)background, wild type C57BL/6, and wild type BALB/c mice can be purchased from Jackson Laboratory (Bar Harbor, ME). $K^{b--/--}D^{b--/--}$ double knockout mice, described previously ??[1]??, can be purchased from Taconic Farms (Germantown, NY). $K^{b--/--}D^{b--/--}CIITA^{--/--}$ triple knock-out and $beta2m^{--/--}CIITA^{--/--}$ double knockout mice can be generated by mating F1 littermates. All our founders were bred for at least eight generations onto a B6 background. Mice should be housed in a specific-pathogen-free colony and fed sterile food (ours was prepared at the Robert Wood Johnson Medical School vivarium). $CD1d^{--/--}$ mice were described earlier. Our $CD1d^{--/-}$ mice of the C57BL/6 or BALB/c background were bred and maintained in the animal facility of Vanderbilt University, Nashville, TN. Antibodies against CD8alpha (53-6.7) and CD49a (DX5, a pan NK cell marker), PE-conjugated antibody against IgG1, and CD1d:Ig dimer can be purchased from BD Biosciences/Pharmingen (San Diego, CA).

Procedure

1 *Sensitization and challenge protocols.* Induction of AAI should be carried out as described elsewhere ??[2]??. Sensitise six to eight-week-old male mice with intraperitoneal injections of OVA (Grade V; Sigma, St. Louis, MO) (100 µg) plus 1 mg aluminum hydroxide (ALUM; Pierce, Rockford, IL) as adjuvant in 0.2 ml PBS on days 0 and 7. On days 14-17, give mice 50 µl of 2 mg/ml OVA in sterile PBS (i.n.). On day 18, 24 h after the last intranasal challenge, harvest bilateral BAL (2 aliquots of 1 ml PBS with 0.6 mM EDTA) and collect tissues for histological analysis. A second protocol can also be utilized as described by Kelly-Welch, et al. ??[3]??. In brief, mice are sensitized with 100 µg OVA and boosted after 14 days. Mice are then challenged via the intranasal route on days 19, 21, 24 and 26. Twenty-

four hours after the last challenge animals are euthanized and BAL fluid collected for determination of cellular infiltration and cytokine levels. Perfused lungs are harvested for histological studies.

2 *CD8+ T cells and NKT cells depletion.* For depletion of CD8+ T cells or NKT cells from C57BL/6, BALB/c, CD1d$^{--/--}$, or K$^{b--/--}$D$^{b--/--}$ knockout mice, inject 100 µg/mouse/week of specific antibody for 4 weeks. Effectiveness can be confirmed by staining splenocytes with CD1d:Ig dimer and CD8-specific antibody.

3 *Allergen-initiated respiratory inflammation.* Measurement of inflammatory mediators can be determined in cell-free BAL fluid (2000 x g, 10 min) by sensitive and specific ELISA, in tandem, for IL-4, IL-5 and IL-13 (R&D Systems, Minneapolis, MN). Resuspend cells in HBSS, enumerate using a hemocytometer, and concentrate onto microscope slides by cytocentrifugation (STATspin, Norwood, MA) (265 x g). Stain cells with Wright–Giemsa stain (Sigma) to determine leukocyte differentials (after counting 200 cells).

4 *Histology.* Euthanize mice by CO_2 inhalation. Remove lungs and embed in paraffin, then cut into 5 to 6 µm sections using a cryostat. Stain the sections with hematoxylin/eosin (H&E) and examine microscopically. Perform mucus staining with PAS. View the sections in a blind fashion, and score disease severity on the basis of cellular infiltration. Stain the paraffin sections with Alcian blue (AB)/periodic acid-Schiff (PAS) stain and observe using standard light microscopy. The mucus-producing cells should be stained dark brown.

5 *Statistics.* Assess data using the Student's *t*-test, ANOVA or Wilcoxon rank sum test, as appropriate. Data should be expressed as mean +/- SEM of evaluations of a minimum of six mice.

References

1. Perarnau, B. *et al. Eur J Immunol.* **29**, 1243-1252 (1999).
2. Das, J. *et al. Nat Immunol* **2**, 45-50 (2001).
3. Kelly-Welch, A.E. *et al. J Immunol* **172**, 4545-55 (2004).

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