Development of Der p 1:IA^b tetramer to determine the importance of Mgl2+ dDCs for the induction of Th2 immunity in response to epidermal painting of house dust mite

Nicole K. Tomm[2], Chen Yao[1], Toshiro Hirai[1], Brian Chicoine[1], Daniel H. Kaplan[1]

[1]Department of Dermatology, Center for Immunology, University of Minnesota, Minneapolis, MN 55455

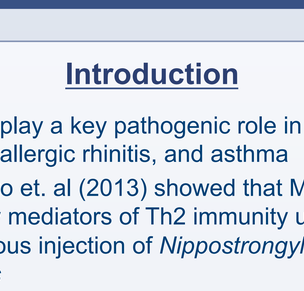
[2]University of Minnesota Medical School, Minneapolis, MN 55455

Introduction

- Th2 cells play a key pathogenic role in atopic dermatitis, allergic rhinitis, and asthma
- Kumamoto et. al (2013) showed that Mgl2+ dDCs are the key mediators of Th2 immunity using subcutaneous injection of *Nippostrongylus brasiliensis*
- Heterozygous Mgl2DTR mice can be used to induce depletion of Mgl2+ dDCs
- House dust mite has been implicated in the pathogenesis of AD
  - Proteolytic activity of Der p 1, one of the major allergens of house dust mite (HDM), plays a role in directing DCs to induce Th2 differentiation

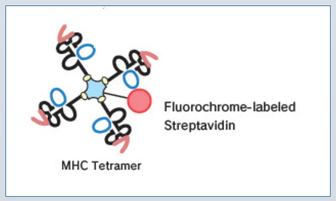
Monomer Purification

- Nickel affinity chromatography
  - Isolates monomer through interaction between Ni^{2+}, which is immobilized in a matrix, and histidine residues
- Monomeric avidin purification
  - Allows for elution of biotinylated protein
- Amicon filtration
  - Removes free biotin to prevent it from competing with monomer to bind to SA-APC



Tetramerization

- Combine monomer and streptavidin conjugated APC at a ratio of 4:1



Tetramer Verification

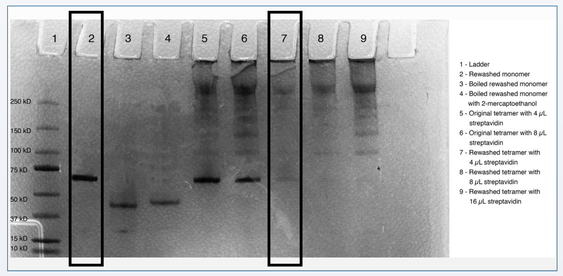


Figure 1: SDS-PAGE verification of Der p 1 monomer and tetramer.

Test Der p 1:IA^b Tetramer with HDM Immunization

- Three treatment groups:
  - No immunization (n=1)
  - 2W1S immunization (10 µg 2W1S+CFA i.p.) (n=1)
  - HDM extract immunization (100 µL HDM +CFA i.p.) (n=1)
- Tetramer pulldown on day 6

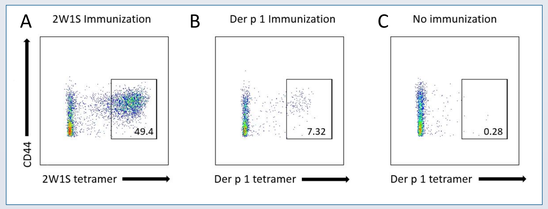
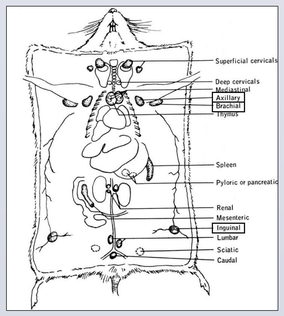


Figure 2: (A) Axillary, brachial, and inguinal lymph nodes and spleen from a 2W1S-immunized mouse treated with 2W1S-PE tetramer. (B) LNs and spleen from a Der p 1-immunized mouse treated with Der p 1-APC tetramer. (C) LNs and spleen from a mouse that was not immunized treated with Der p 1-APC tetramer.



Identification of Der p 1-specific T Cells After Epidermal Painting of HDM

- Three treatment groups of Mgl2-DTR mice:
  - No DT/no HDM (n=2)
  - No DT/epidermal painting of HDM (n=3)
  - DT/epidermal painting of HDM (n=3)

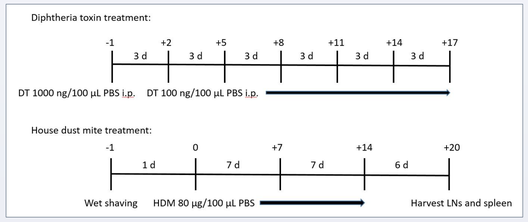


Figure 3: Schematic showing the schedule of diphtheria toxin injections and house dust mite epidermal painting.

Results

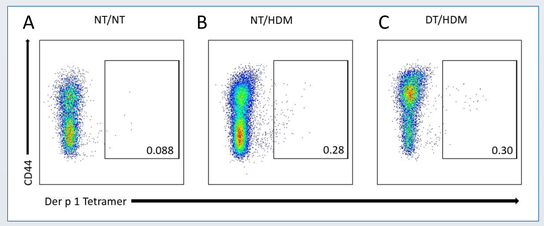


Figure 4: (A) Axillary, brachial, and inguinal lymph nodes and spleen from a mouse that did not receive DT injection or HDM treatment. (B) LNs and spleen from a mouse that did not receive DT injection but did receive HDM treatment. (C) LNs and spleen from a mouse that received DT injection and HDM treatment.

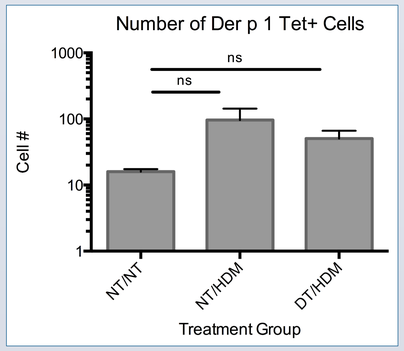


Figure 5: Average number of Der p 1 tetramer positive cells in each treatment group. t-test was performed to assess if there were statistically significant differences between groups.

Key Findings

1. Der p 1 monomer was able to be purified from Drosophila S2 cell supernatant and was able to form tetramer with SA-APC
2. Der p 1 tetramer was able to identify Der p 1-specific CD4+ T cells after priming with HDM+CFA.
3. It could not be determined whether Mgl2+ dDCs are important for the induction of the Th2 response after epidermal painting with HDM due to minimal immune response in the NT/HDM treatment group
   - There was no statistically significant difference in the number of tetramer positive cells between any of the groups
   - Phenotype analyses could not be performed because there were too few cells for the results to be trustworthy

Discussion

- Repeated painting of HDM could have caused Der p 1-specific T cells to migrate to the skin instead of staying in the lymph node
- Amount of antigen used is too low
- Other concerns:
  - Lack of intradermal injection of HDM treatment group
  - • B6 mice have less robust Th2 responses compared to Balb/c mice
  - Langerhans cells were also depleted in epidermis but not lymph nodes of Mgl2DTR mice

Future Directions

- Increase number of applications of HDM
- Cross Mgl2DTR B6 mice and WT Balb/c mice and use F1 generation for experiments
- Include HDM intradermal injection group
- Use bone marrow chimeras = transfer bone marrow from Mgl2DTR mice into WT Balb/c mice so there are Mgl2+ dDCs that express DTR as well as WT Langerhans cells

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