Leukocyte population dynamics in response to ovalbumin peptide immunization in DO11 mice

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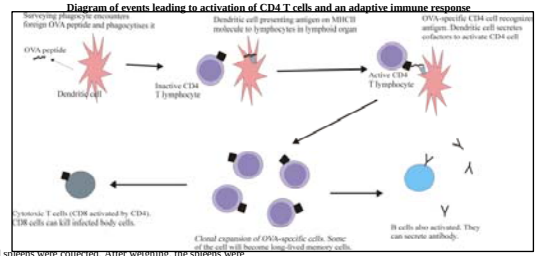
Introduction

One of the goals of immunology is to understand in detail the way the immune system works in order to design new therapies against disease. Vaccination is a good example of how the immune system can be harnessed to provide protection against pathogens even before these are encountered. However, our knowledge of the workings of the immune system is still limited and the response to vaccines which have been in use for the past 50 years are still not fully understood. We used a simplified method with a single "vaccine" molecule (ovalbumin peptide, OVA) to identify population changes in mice. **Our hypothesis is that OVA would start an immune response involving mostly the CD4 T lymphocytes and also possibly activation of B cells with antibody secretion. We also anticipated the establishment of OVA-specific memory cells over the course of several weeks following immunization.** The project presented here aims to follow the immune response to OVA in normal mice of the DO11.10 background. The data is meant to show the normal response patterns and serve as a control to future work in PECAM deficient mice.

On encountering antigen, specialized phagocytes like macrophages and dendritic cells (DCs) endocytose it and become activated. They then travel to lymphoid organs (lymph nodes and spleen) where they prime the adaptive arm (T and B lymphocytes) of the immune response. T and B lymphocytes are capable of specifically recognizing various antigens, but in order to do so, they must first be primed by antigen-presenting phagocytes. Following priming, they will proliferate into a clonal population that maintains the same antigen specificity. B and T lymphocytes are also responsible for the establishment of immune memory, being capable of responding very quickly upon encounter with the same antigen again.
The molecular basis for specific recognition lies in the T cell receptor (TCR) for T lymphocytes and in the B cell receptor for B lymphocytes.

The DO11.10 strain is a TCR transgenic strain capable of specifically responding to ovalbumin (OVA). In order to generate a response, mice must first be primed with ovalbumin peptide. A few days later, a small pre-existing population of OVA-specific lymphocytes would have been primed and expanded, modeling a simple immune response. The OVA-specific lymphocytes generated can be easily tracked with antibodies.

Diagram of events leading to activation of CD4 T cells and an adaptive immune response

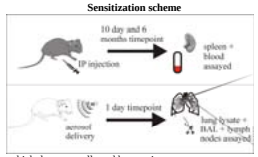


Methods and materials

Mouse strains used:
- DO11.10 TCR-transgenic on C57/B10 background (black mice)
- DO11.10 TCR-transgenic on FVB/n background (white mice)

OVA sensitization (intra-peritoneal):
Mice were sensitized to ovalbumin. The mice were sacrificed and blood and spleens were collected. After weighing, the spleens were minced and passed through a 70 um nylon mesh to obtain a single cell suspension. Blood was enriched for leukocytes by a quick red blood cell lysis with water. Timepoints assayed were at 10 days and at 6 months.

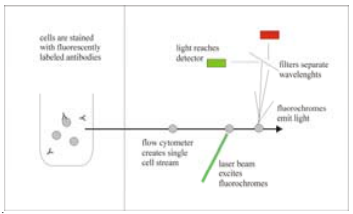
Sensitization scheme



OVA sensitization (inhalation):
10 ug purified peptide were suspended in 1 ml water and delivered to the lungs as a fine mist. The immediate response was measured at the 24 hour timepoint. Mice were sacrificed and the lungs, broncho-alveolar lavage (BAL) and mediastinal lymph nodes collected. The lungs and lymph nodes minced and digested in collagenase for 30 min. The tissues were then passed though a 70 um nylon mesh to obtain a single cell suspension. Residual RBC contamination was removed by a short water-lysis step.

Antibody panels used and flow cytometry
Leukocyte populations in the samples collected were assayed by flow cytometry. Samples were surface stained with various antibody panels, after which data was collected by running on on a DakoCytomation Cyan ADP machine. Files were analyzed on Summit software (Dako).

Cell population analysis by flow cytometry



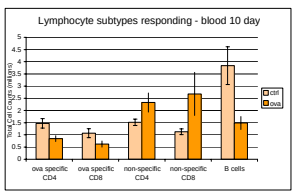
Antibody panels used

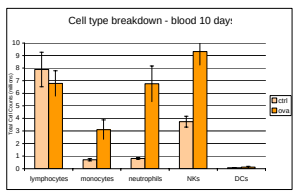
T cells:	B cells:	Granulocytes/ monocytes/ DCs:	NKs:
•CD4/8 (T lymphocyte subtype)	•CD19 (B cell marker)	•CD11b (granulocyte, macrophage and DC expressed integrin)	•CD3 (T lymphocytes and NK T cells)
•DO11 (ovalbumin specificity)	•IgD (naive cell marker)	•F4/80 (monocyte/ macrophage subtype)	•CD49b (Natural Killer cell marker)
•☐CD62L (naive cell marker)	•CD62 (naive cell marker)	•CD11c (DC marker)	
•CD44 (memory/effector marker)/CD3		•CD115 (monocyte subtype)	
		•Gr-1 (myeloid lineage marker)	

Data analysis
Statistical significance was established using the Tukey-Kramer test for means on JMP software (SAS Institute Inc). P values of 0.05 were considered significant.

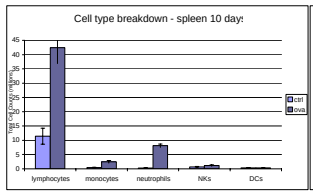
Results: leukocyte dynamics in response to intra-peritoneal OVA - 10 days post-administration

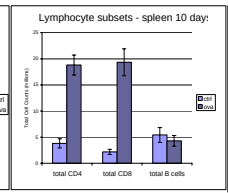
- Blood leukocyte counts increased significantly in response to OVA
- The increase was due to recruitment of monocytes and neutrophils (innate immunity)
- B cells showed a significant decrease from blood (below)

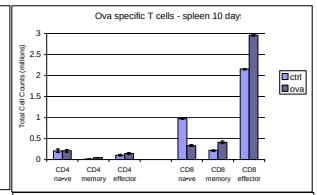


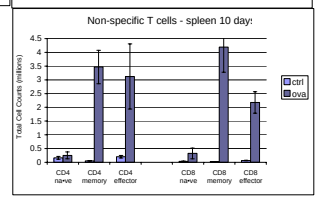


- Spleen was enlarged, with significant increase in cellularity and weight
- Lymphocytes showed proliferation in the spleen (opposite, top)
- Response from both CD4 and CD8





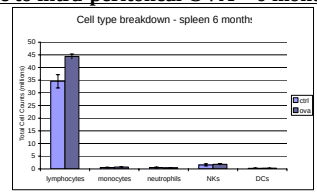


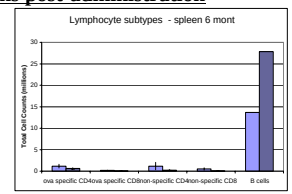


- Proliferating cells were of memory and effector phenotype
- Non-specific lymphocytes proliferated more than OVA-specific cells (below)

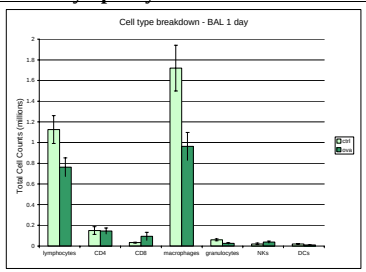
Results: long-term dynamics due to intra-peritoneal OVA – 6 months post-administration

- Blood counts returned to baseline
- Circulating cell subtypes same as controls; innate immune effectors disappeared
- Spleen weight returned to normal but cellularity remained elevated
- Increased numbers of B cells responsible for higher cell counts in treated mice
- No OVA-specific T cell memory population found





Results: lymphocyte mobilization following aerosolized OVA inoculation – 1 day post-administration



- CD8 lymphocytes showed a statistically significant increase in broncho-alveolar lavage
- Both ova-specific and non-specific CD8 lymphocytes showed proliferation.
- Unexpectedly, did not show a similar increase
- DCs and resident lung macrophages decreased in BAL and were not sequestered by lung tissue or lymph nodes

Conclusions

- Ovalbumin peptide elicits both innate and adaptive immune responses
- Lymphocyte proliferation occurs in the spleen at 10 days following immunization with intra-peritoneal ovalbumin in CSF
- Likely due to cross-presentation, CD8 lymphocytes are also activated
- B cells show a response at early timepoints, followed by the establishment of a memory population in the spleen following IP immunization. It is possible that this response is oriented against ovalbumin, but the CFA is also highly immunogenic and could presumably elicit a response
- Despite having good markers for isolation of OVA-specific lymphocytes, we failed to detect a CD4 or CD8 memory population.
- In the lung, activation occurs very quickly, followed by infiltration of effector CD8s belonging to the adaptive arm of the immune system.

References

Murphy KM; Heimberger AB; Loh DY. 1990. Induction by antigen of intrathymic apoptosis of CD4+CD8+TCRlo thymocytes in vivo. Science 250:1720-3