Leukocyte population dynamics in response to ovalbumin peptide immunization in DO11 mice
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Introduction
One goal of immunology is to understand 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. There are numerous. 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 simple method with a single “carrier” peptide to confirm populations of DO 11+ T cells and populations 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.

Methods and materials

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

OVA sensitization (intraperitoneal):
The mice were sacrificed and blood obtained from the heart. The spleens were excised and weighed and then put on ice. Freshly isolated lymphocytes were obtained to obtain a single cell suspension. Blood was isolated from leukocytes by a quick and efficient lysis with water. Timepoints assayed were at 10 days and at 6 months.

OVA sensitization (inhalation):
Residual RBC contamination was removed by a short water-lysis step. Antibody panels used and flow cytometry

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
- At the lung, activation occurs very quickly, followed by infiltration of effector CD8 cells belonging to the adaptive arm of the immune system.

References


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