

Labs 9, 10
Immunohistochemistry

Lab 9: cutting sections

Lab pairs will meet in my research laboratory at the assigned times to cut sections of frozen mouse spleen using the cryostat.

Lab 10: staining sections

Immunohistochemistry allows us to visualize the positions of particular cells within a tissue microenvironment. The technique is analogous to the ELISAs we've done, in that antigens are detected using enzyme-conjugated antibodies. The difference is that the antigens we're targeting are part of thin tissue sections, and the developing reagents produce insoluble colored precipitates so that the stain marks only the cells bearing those antigens.

Prior to this afternoon's lab:

Last week, we cut mouse spleens into 7 μm thick sections using the cryostat. The sections were fixed in acetone to preserve the native structure of the antigens to be recognized by antibodies, then frozen.

Blocking

This morning, tissue sections were thawed and circled with a "PAP pen", which leaves a hydrophobic barrier around each section. Sections were rehydrated in PBS (phosphate buffered saline) for 20 minutes. Sections were then incubated with 1% BSA supplemented with rat and goat serum, to block any "sticky" sites on the tissue that might bind your labeled antibodies non-specifically.

Materials

Each team will get

1. their slide containing two spleen sections, rehydrated and blocked
2. Conjugated reagent solutions:
 - a. goat anti-mouse CD3-biotin
 - b. goat anti-mouse IgM-AP
 - c. streptavidin-HRP
3. "humid box" in which to incubate and develop sections. You will need to wet the paper towels in the bottom to create the humid environment.
4. Washing solution: 1% BSA in PBS with 0.05% Tween-20 detergent
5. AP developing reagent, HRP developing reagent

Staining

**Be careful not to touch the spleen section with your pipet tip; this will gouge it.

1. Gently tap slide on its side (onto a paper towel) to remove excess 1% BSA solution.
2. Add 100 ul anti-mouse CD3-biotin + anti-mouse-IgM-AP to section #1
Add 100 ul anti-mouse CD3-biotin to section #2
3. Incubate 30 minutes at room temp in a humidified box.
4. Tap slide on its side to remove excess Ab solution. Wash 3 times in BSA solution with shaking, 5 minutes/ wash. Tap off excess BSA solution.
5. Add 100 ul SA-HRP solution to both sections.
6. Incubate 30 minutes at room temp in humidified box. Wash 2x in BSA, 5 minutes/wash; then immerse briefly in PBS.

Developing

7. Add 100 ul AP developing reagent to section #1 (put 100 ul PBS on section #2).
Incubate slide at room temperature. Check at 1-minute intervals to see how development of blue stain is progressing.
8. When blue color development is sufficient, rinse slide in PBS to stop the reaction.
9. Add 100ul HRP developing solution to both section #1 and #2
When red stain development is sufficient, rinse slide in PBS to stop the reaction.
10. Place 100 ul of PBS on each section to prevent drying while making your observations.

Diagram your stained spleen section, and label the zones/components (refer to Figure 1.23 in your text for regions).

Questions (you may refer to the Parham text for help with these)

1. How does the architecture of the spleen compare to that of the lymph node?
2. How does the function of the spleen compare to lymph node?
3. How do antigens enter the spleen? How do effector lymphocytes leave the spleen?