An Investigation of Lymphocyte Development

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Summary:

Every cell in an organism arises from one stem cell. Cells express different genes, or differentiate , in order to carry out the remarkable variety of functions necessary for survival.

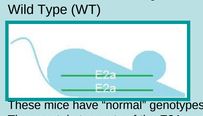
The cells of the immune system are programmed to defend the organism from damage by pathogens. B-cells respond to pathogens by secreting antigen specific antibodies, and T-cells kill infected cells or respond to cells that have been infected. These functions are vital in the homeostasis of the human body. When errors occur in the development of B or T cells, autoimmune diseases and leukemic cancers may result.

How does a pluriponent cell become such a specialized part of the immune defense? What changes in development cause disease? In response to these questions, various assays have been developed to determine what chemical signals a cell has been exposed to and what type of functions it can currently perform.

Mice:

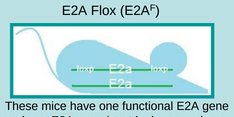
Mice are a model system to study mammalian lymphocyte cell development. In order to research the role the E2A gene plays in cell differentiation, a few distinct mice lineages are studied:

Wild Type (WT)



These mice have "normal" genotypes. They contain two sets of the E2A genes.

E2A Flox (E2A^{F})

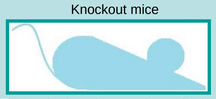


These mice have one functional E2A gene and one E2A gene inset by lox p markers. When Cre is induced, one E2A gene will stop functioning.

E2A Flox (E2A$^{F/F}$)

These mice have both E2A genes inset by loxp markers. When Cre is induced, their E2A genes will stop functioning.

Knockout mice

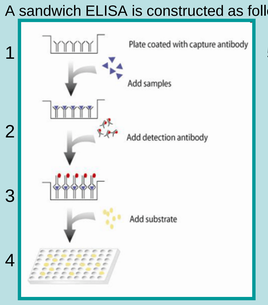


These mice have been engineered to have deleted or entirely dysfunctional E2A genes.

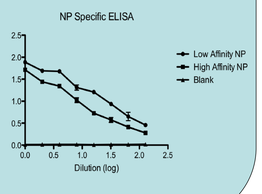
ELISA:

The enzyme-linked immunosorbent assay is used to detect the presence of an antibody in a sample.

A sandwich ELISA is constructed as follows:

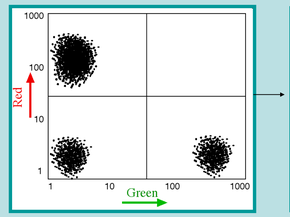


5 Measure the color concentration of the assay. Graphically record results.

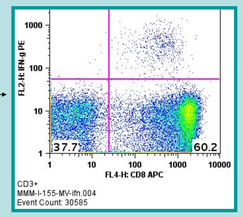


FACs:

Flourescence Activated Cell Sorting is a mechanized process by which cells are sorted by the proteins they express. Cells are tagged with antibodies linked to a fluorescent die (flourochromes). If they have the protein in question, they will emit a specific wavelength when excited by a laser.



Photosourse: http://www.bio.davidson.edu/



Photosource: microbiology.emory.edu/

The machine then graphically organizes the cells by which wavelength they emit.

Programs like Flow Jo can further illustrate cell expression populations

Genotyping

1.Cell Extraction

Start with: mouse cells from the tail, ear, bone marrow or spleen
Grind or sonicate to open cells
Add a detergent to remove membrane lipids
Add a protease to remove proteins
Result: Extracted DNA!

2. PCR

Polymerase Chain Reaction

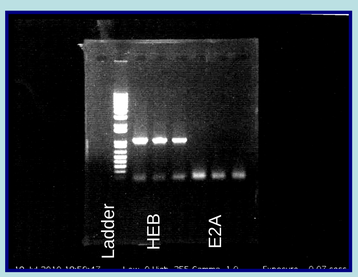
Start with: Extracted DNA
Disrupt bonds between DNA strands
Let polymerase attach to each strand
Polymerase puts dNTPs together to make more strands
Result: Thousands to millions of one strand of DNA!

3. Electrophoresis

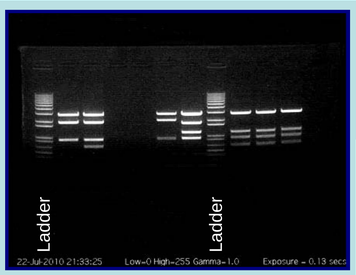
Start with: Thousands to millions of one strand of DNA
Make a gel with agarose, ethidium bromide, and buffer
Dye DNA and suspend in the gel
Run a electric current through the gel
DNA is negatively charged and will move towards the positive charge.
Heavier strands move slower and lighter strands move faster.
Result: A DNA "ladder"!

4. Comparison

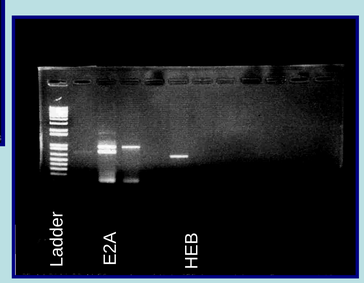
Start with: A DNA ladder
Compare to an established DNA ladder
Result: a known genotype!



Gel run for mice genotyping



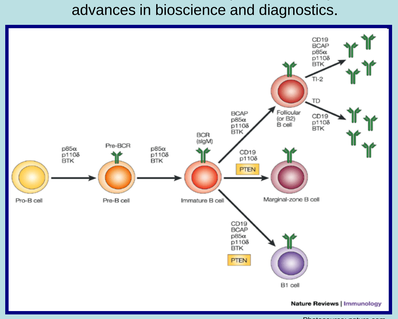
Gel run for use in molecular cloning.



Gel run for mice genotyping

Conclusion:

The ELISA, FACs plotting, and genotyping procedures are key to our current understanding of the lymphocyte development pathway. With years of extensive research our knowledge of this process of differentiation will increase .The study of the mouse's model mammalian immune system has the potential to spark great advances in bioscience and diagnostics.



Photosource: nature.com

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