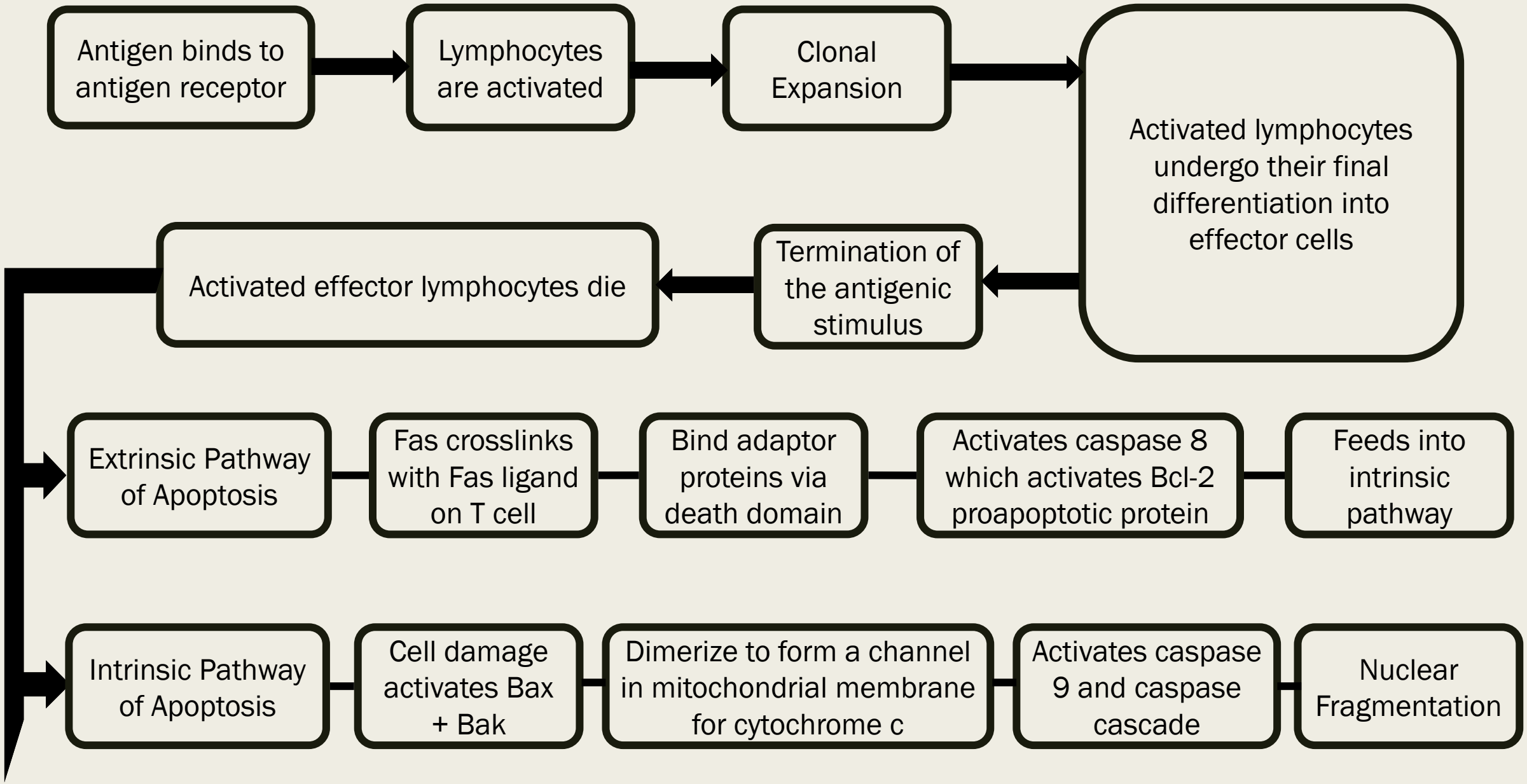
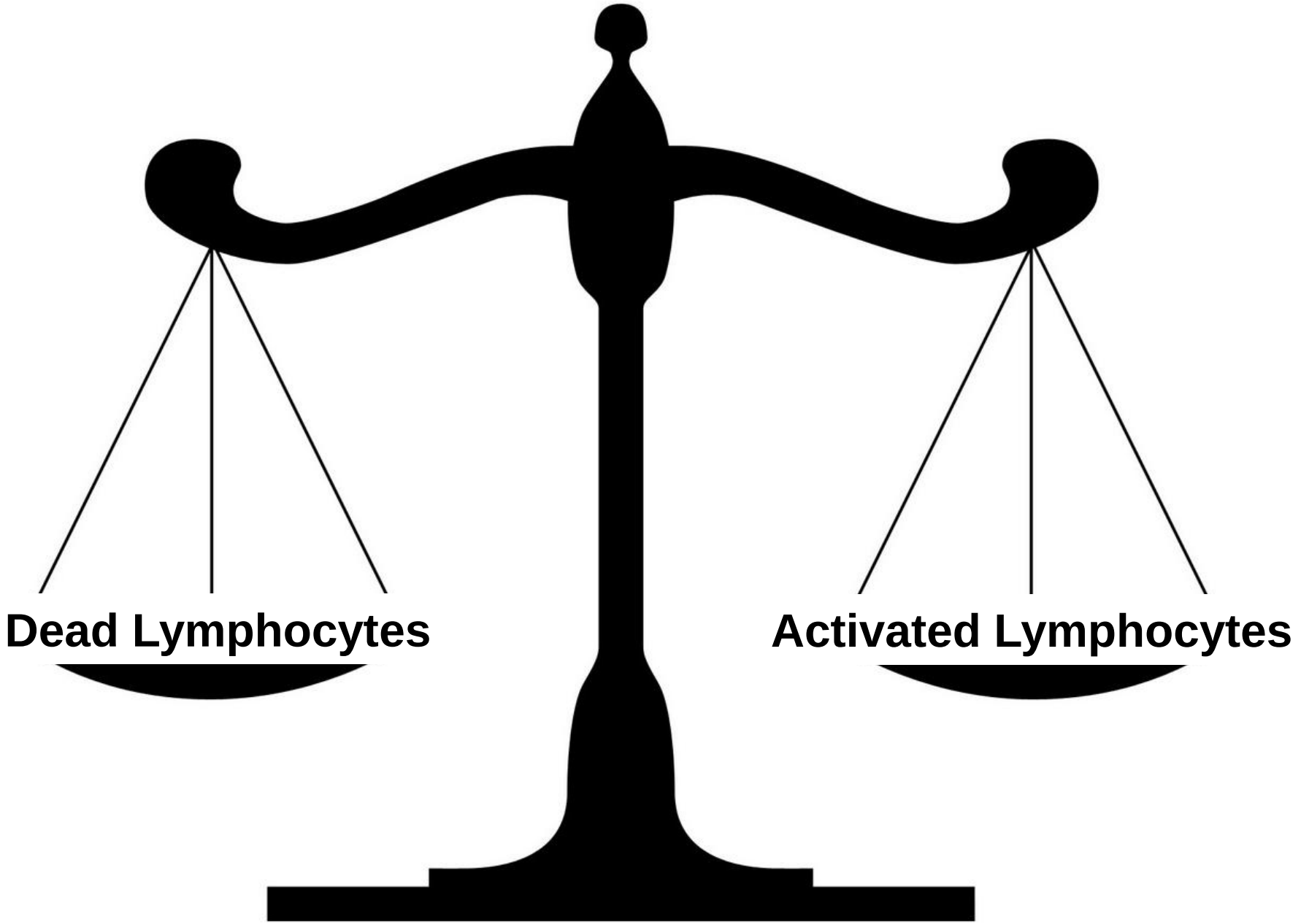
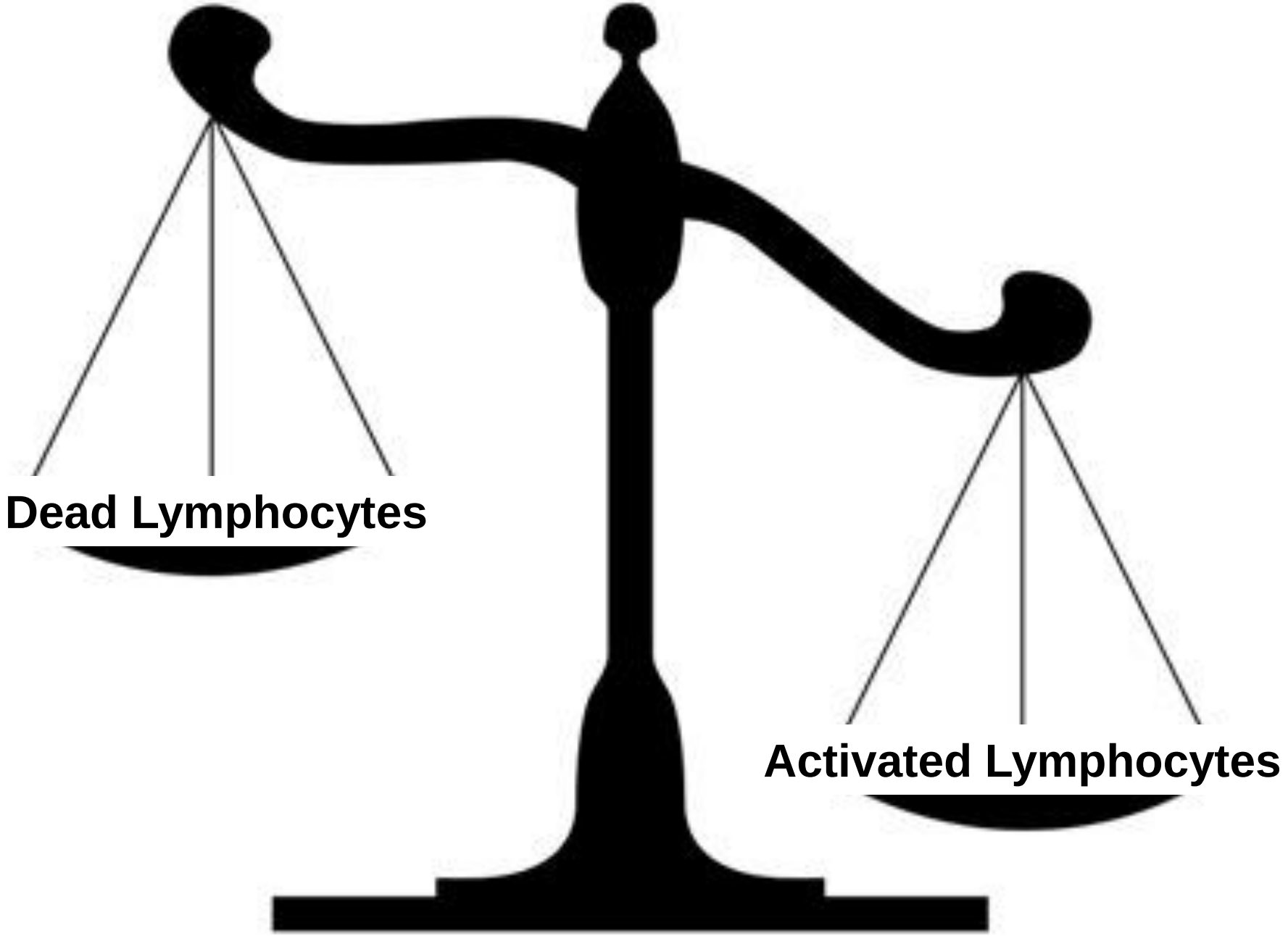
AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS)

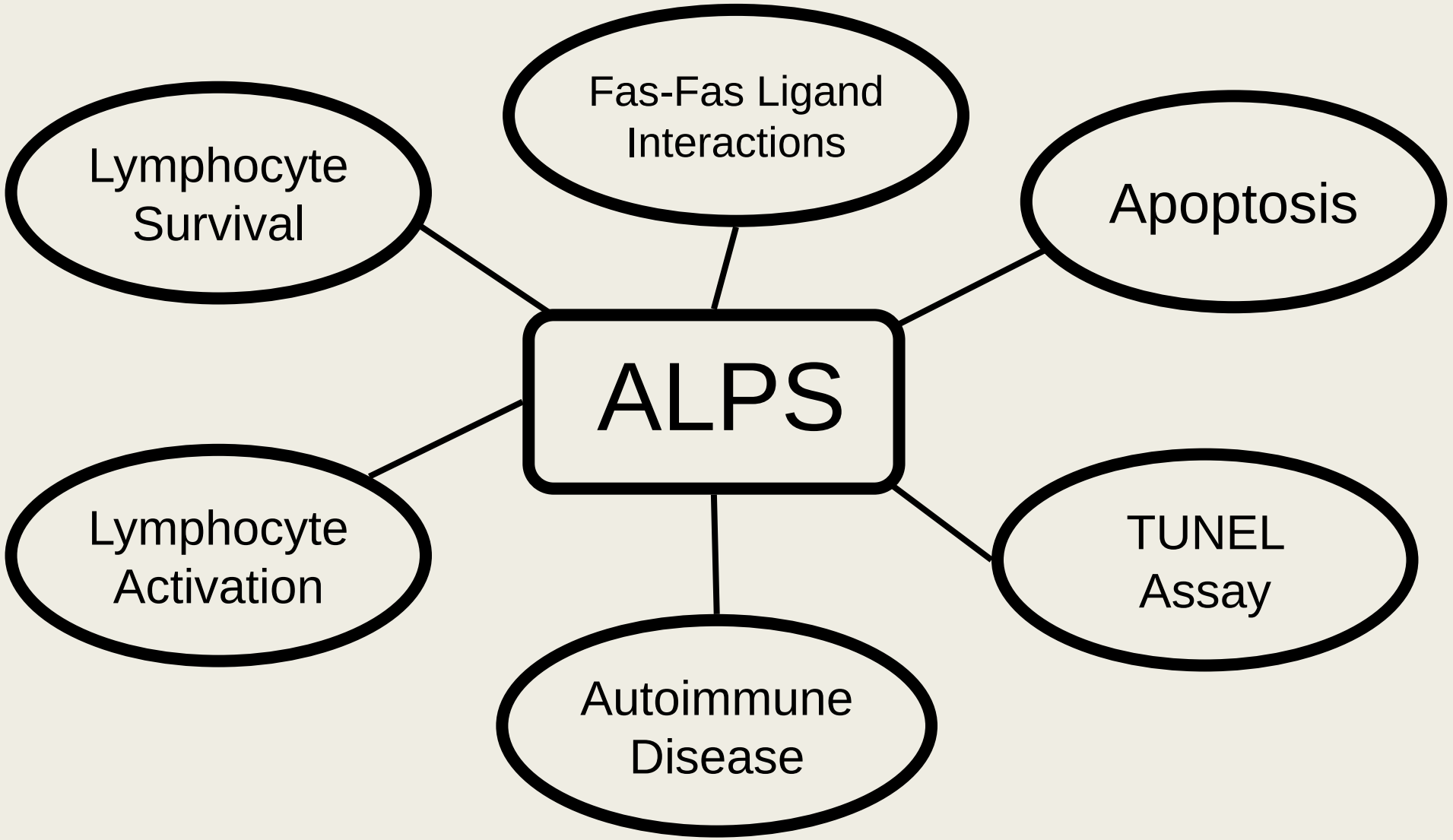
Amer M. Sawalha


Antigen binds to antigen receptor
Lymphocytes are activated
Clonal Expansion
Activated lymphocytes undergo their final differentiation into effector cells
Termination of the antigenic stimulus
Activated effector lymphocytes die
Extrinsic Pathway of Apoptosis
Fas crosslinks with Fas ligand on T cell
Bind adaptor proteins via death domain
Activates caspase 8 which activates Bcl-2 proapoptotic protein
Feeds into intrinsic pathway
Intrinsic Pathway of Apoptosis
Cell damage activates Bax + Bak
Dimerize to form a channel in mitochondrial membrane for cytochrome c
Activates caspase 9 and caspase cascade
Nuclear Fragmentation

Autoimmune lymphoproliferative syndrome (ALPS) is characterized by dysregulation of the immune system, due to an inability to regulate lymphocyte homeostasis through the process of lymphocyte apoptosis (a form of programmed cell death).


Dead Lymphocytes
Activated Lymphocytes


Dead Lymphocytes
Activated Lymphocytes


Fas-Fas Ligand Interactions
Lymphocyte Survival
Apoptosis
ALPS
Lymphocyte Activation
TUNEL Assay
Autoimmune Disease

ALPS is the first disease known to be caused by a primary defect in programmed cell death.

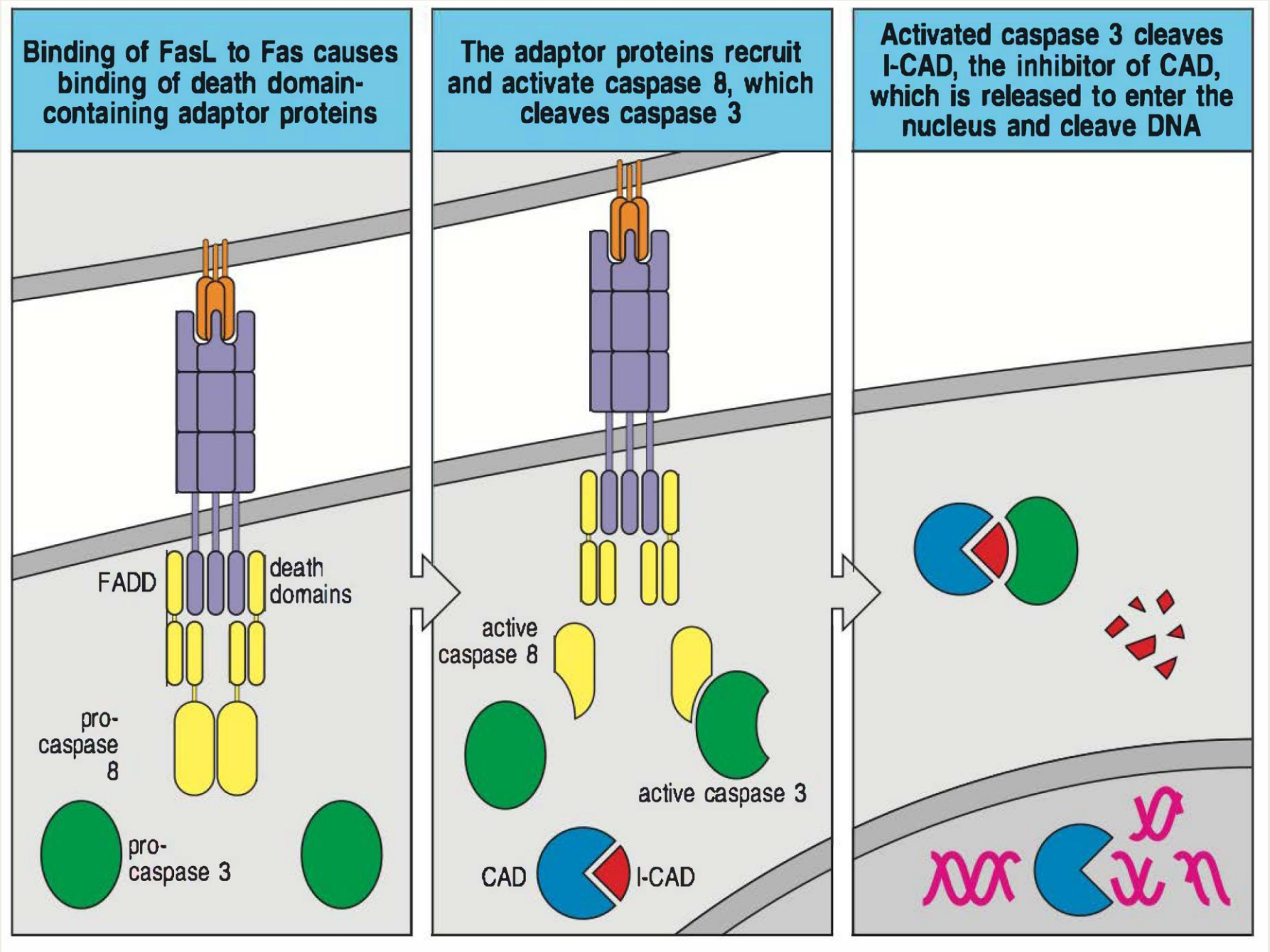
It is also the first autoimmune disease with a defined genetic basis.

It is estimated that the number of ALPS cases worldwide is a bit over **500**.

However, its incidence and prevalence are still…UNKNOWN

Genetics

- Most patients are heterozygous for a dominant mutation in the FAS gene.
- In some cases, ALPS is due to somatic mutations of FAS that occur in an early lymphoid progenitor.
- **The proportion of lymphocytes carrying the somatic mutations may increase over time due to defective apoptosis.**
- Activated T cells do not undergo Fas-mediated apoptosis.


Binding of FasL to Fas causes binding of death domain-containing adaptor proteins
The adaptor proteins recruit and activate caspase 8, which cleaves caspase 3
Activated caspase 3 cleaves I-CAD, the inhibitor of CAD, which is released to enter the nucleus and cleave DNA
FADD
death domains
pro-caspase 8
pro-caspase 3
active caspase 8
active caspase 3
CAD
I-CAD

Clinical Characteristics

- **Non-malignant lymphoproliferation** (lymphadenopathy, hepatosplenomegaly with or without hypersplenism) that often improves with age.
- **Hypergammaglobulinemia**
- **B-cell lymphocytosis**
- **Lifelong increased risk for lymphomas**.
- **Autoimmunity** may result because Fas-mediated killing is a mechanism for removing auto-reactive B cells.

Diagnosis

- Patients with ALPS due to FAS mutations have elevated serum levels of:

1. FasL
2. IL-10
3. Vitamin B_{12}

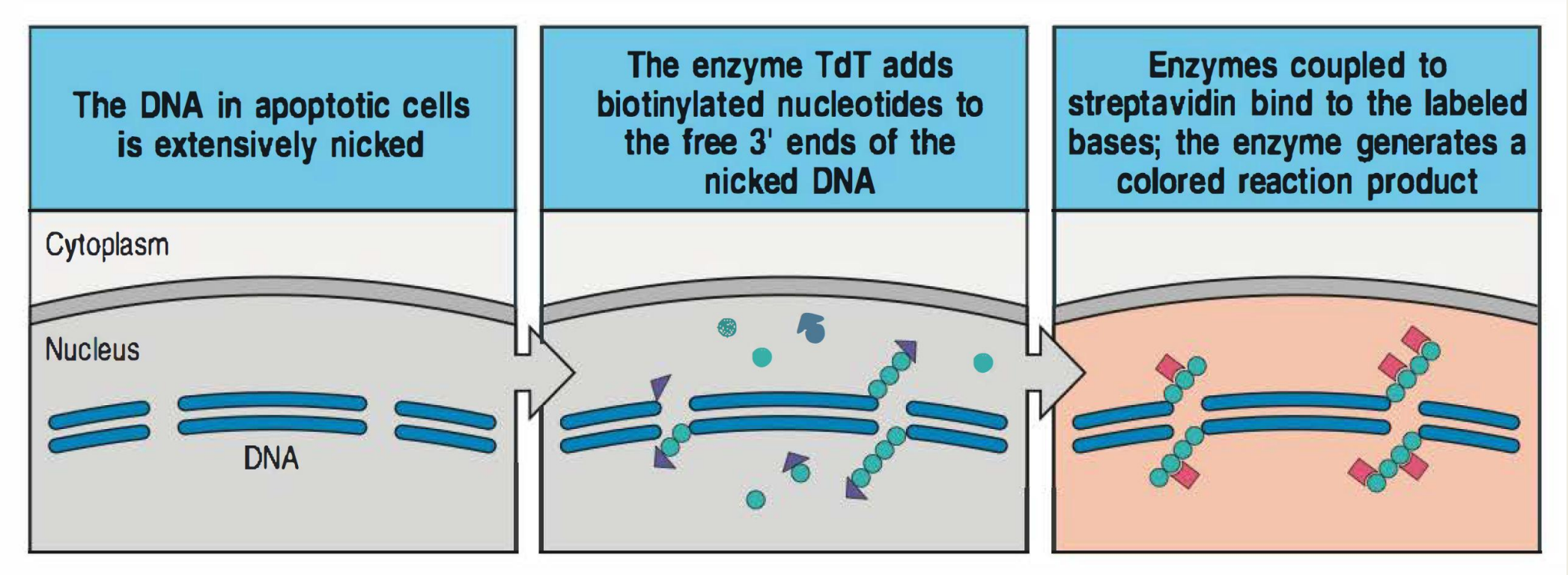
} RELIABLE BIOMARKERS

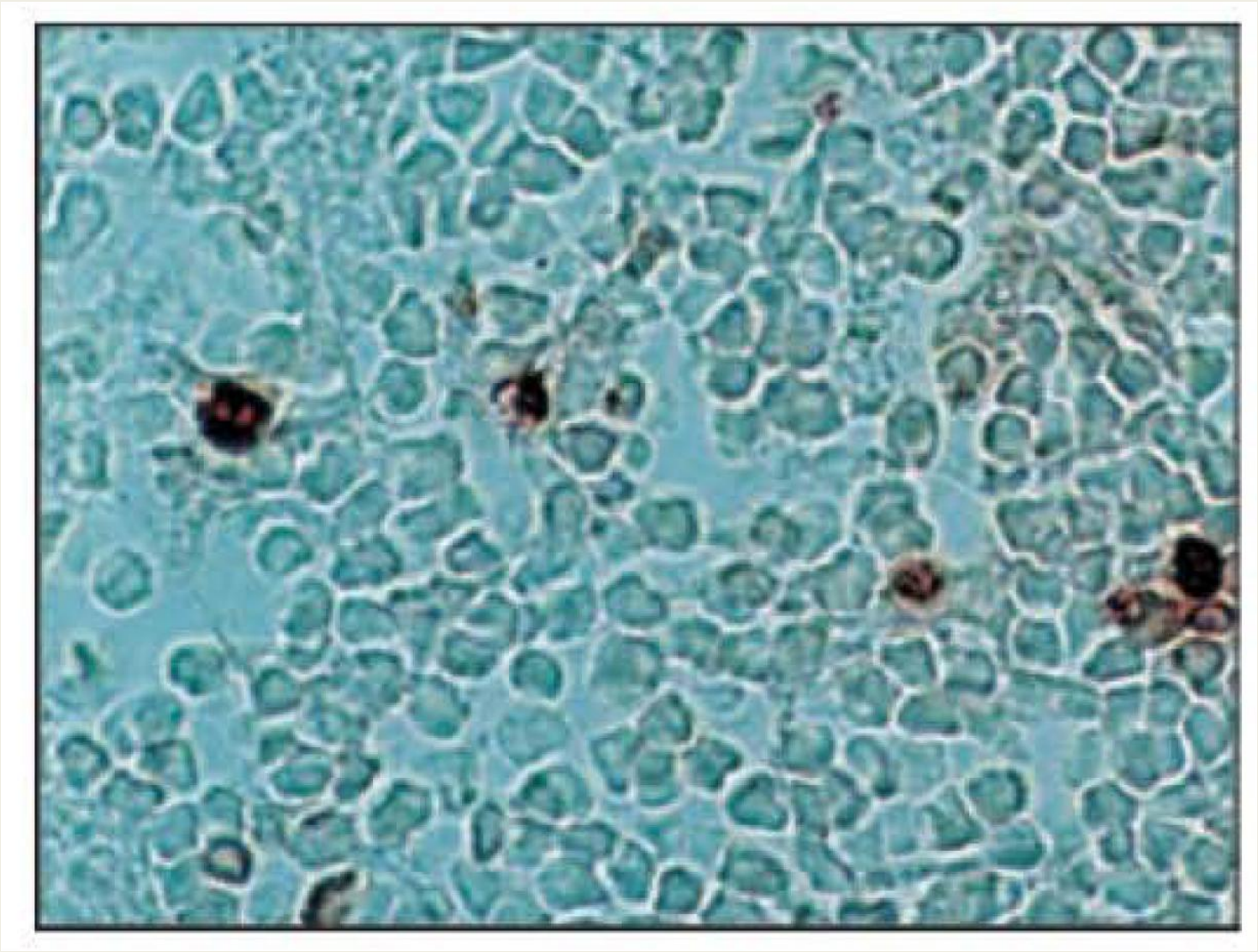
- Patients also present with an **increase in DN T lymphocytes.**

Diagnosis

- TUNEL assay (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) – uses activity of the terminal deoxynucleotidyl transferase enzyme to label the 3’ ends of DNA strand breaks which may then be identified by light microscopy or flow cytometry.
- TUNEL assay (immunodetection) detects breaks in DNA as cells undergo apoptosis

TUNEL Assay


The DNA in apoptotic cells is extensively nicked
The enzyme TdT adds biotinylated nucleotides to the free 3' ends of the nicked DNA
Enzymes coupled to streptavidin bind to the labeled bases; the enzyme generates a colored reaction product
Cytoplasm
Nucleus
DNA



Treatment

- Corticosteroids
- Immunosuppressants
- Splenectomy (reserved for severe cases)

The Case of Ellen O’Hara

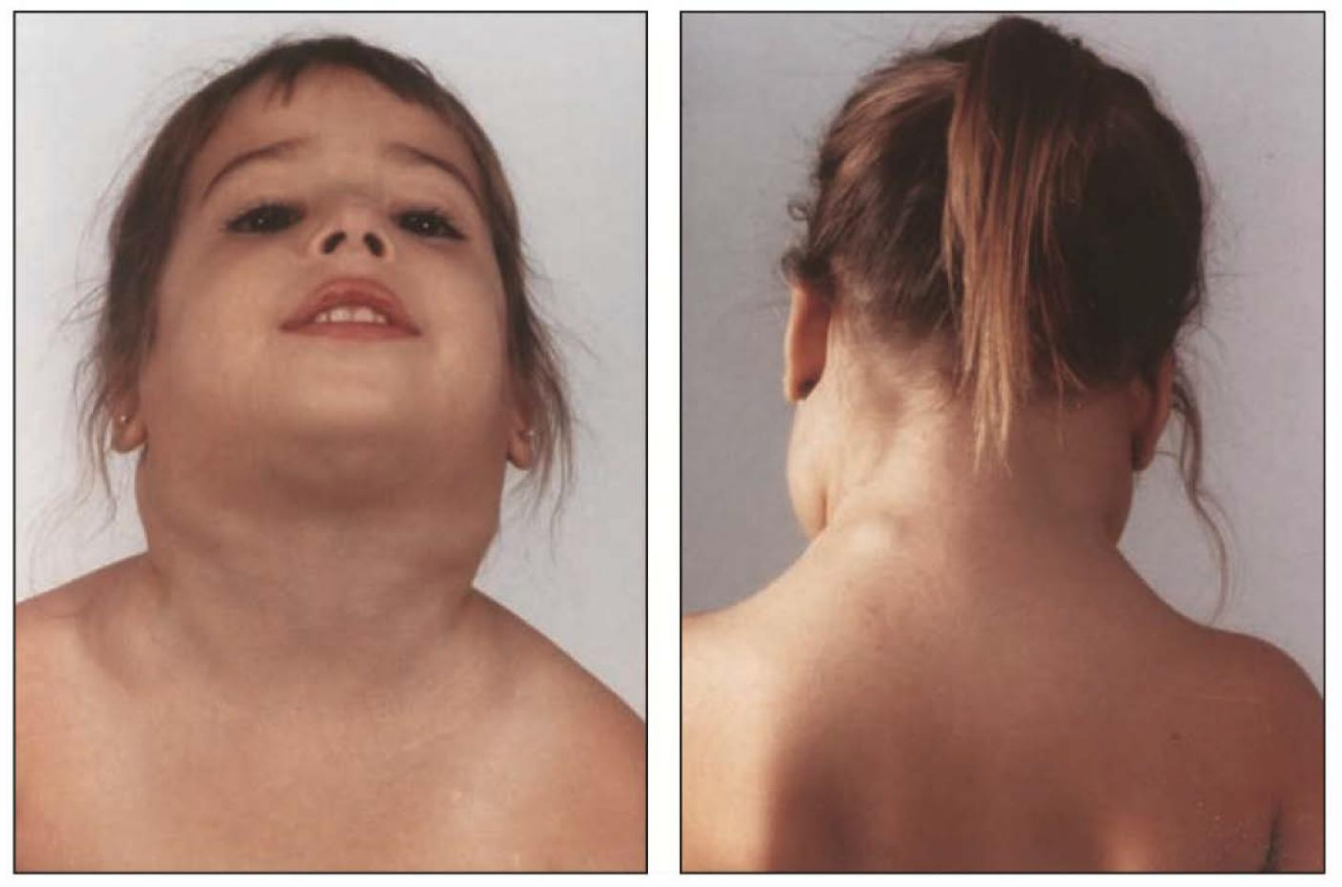
Ellen was breastfed. She received her routine immunizations without any adverse reactions. She had no unusual infections and seemed to be growing and developing normally.

Ellen was born after a normal uncomplicated pregnancy.

18 Months

During a routine check-up, her pediatrician discovered that she has splenomegaly and lymphadenopathy.

The Case of Ellen O’Hara



The Case of Ellen O’Hara

Lab tests revealed:

1. WBC count = 12,500/μL
2. Lymphocyte count = 9175/μL (high)
3. IgG = 4000 mg dl^{-1}
4. IgM = 400 mg dl^{-1}
5. IgA = 1660 mg dl^{-1}

} Hypergammaglobulinemia

The Case of Ellen O’Hara

- Flow cytometry of her lymphocytes displayed an **increase in the number of double negative T lymphocytes and B cells**.
- The vast majority of her T cells expressed TCRαβ and CD3 which is a component of TCR.
- A small percentage of her T cells expressed CD4 and CD8.

The Case of Ellen O’Hara

- Biopsy of a lymph node showed extensive enlargement of the follicles.
- There was a **marked increase in the numbers of immunoblasts and plasma cells in the paracortical region.**

The Case of Ellen O’Hara

- No infection
- No chromosomal abnormality
- No oligoclonality of TCR
- No malignancy
- Therefore, Ellen received **prednisone** and **cyclosporin A**. Her lymph nodes rapidly reduced in size after this therapy.

The Case of Ellen O’Hara

18 Years

- Size of lymph nodes decreased spontaneously.
- Autoantibody against platelets
- Thrombocytopenic purpura.
- Platelet count = 75,000 μL^{-1}
- Treated with dexamethasone

32 Years

- Neutropenia
- Autoantibody against granulocytes
- Neutrophil count = <1000 μL^{-1}

The Case of Ellen O’Hara

Family history:

1. Paternal grandfather had **splenomegaly** and generalized **lymphadenopathy** as a child. His **spleen was removed** at age 25. At age 60, he developed **B-cell lymphoma**.
2. Father had splenomegaly and lymphadenopathy but no clinical symptoms.
3. Mother, brother, and maternal grandparents had normal T cells.

The Case of Ellen O’Hara

TUNNEL assay was performed on blood mononuclear cells from Ellen, her parents and her paternal grandfather.

1. The cells were first stimulated in vitro with PHA for 3 days.
2. Growth of the resulting T cell blasts was continued for 3 weeks **by the addition of IL-2**.
3. Half of the cultures were exposed to an antibody to Fas.
4. The percentage of cells undergoing apoptosis was then counted using TUNNEL assay.

The Case of Ellen O’Hara

- Normally 35-70% of cells should demonstrate apoptosis.
- Results:

1. 60% of mother’s cells
2. <1% of father’s cells
3. 2% of Ellen’s cells
4. 1.4% of grandfather’s cells

The Case of Ellen O’Hara

- The FAS and FASL genes were examined in DNA samples from Ellen, her father and paternal grandfather.
- An identical single base transconversion, causing a premature termination codon, was found in one of the alleles of the FAS gene in the samples.

Question 1

Patients with ALPS are heterozygous for the mutation in FAS or FASL; they have one normal allele and one mutant allele. How do you explain the dominant inheritance?

Fas and FasL are homotrimer signaling complexes.

If 1 of the elements of this complex is mutant, the timer is rendered ineffective.

This is referred to as “dominant negative”.

Question 2

Ellen's great-aunt (her paternal grandfather's sister) was found to have the same FAS mutation as Ellen, yet she had no symptoms. How can this be explained?

Some people will not show any clinical symptoms, but there would be in vitro impaired lymphocyte apoptosis.

Environmental and genetic factors play a role in the full expression of a genetic disease like ALPS.

This is referred to as “variable expression”.

Question 3

It is advantageous for viruses to inhibit apoptosis so that the host cells in which they thrive do not get eliminated by apoptosis induced by recognition by cytotoxic T cells. How might a virus accomplish this?

EBV produces a protein similar to Bcl-2 which renders infected cells resistant to cytotoxic T cells.

HSV has 2 genes (Us3 and Us5) which encode proteins that inhibit caspases.

Question 4

When Fas is activated by FasL it associates with and activates caspase 8. When the gene encoding caspase 8 is knocked out in mice, this proves to be lethal at the fetal stage. Would it be worthwhile to search for caspase 8 mutations in patients with ALPS when there is no mutation in FAS or FASL?

YES, a point mutation in the gene encoding caspase 8 would interfere with the Fas complex.

Thus, a missense mutation in the caspase 8 gene would cause ALPS.

Thank you