LYMPHOPOIESISBANDT (EXCLUDING IMMUNOPOIESIS)

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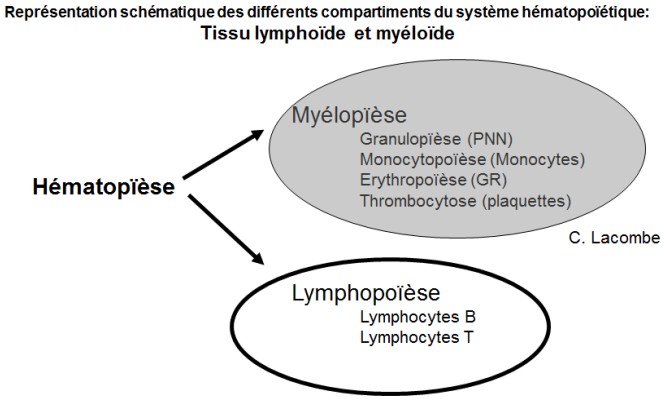
1. INTRODUCTION

Hematopoiesis is a hierarchical, stepwise model of differentiation where the most immature cells result in the most mature cells.

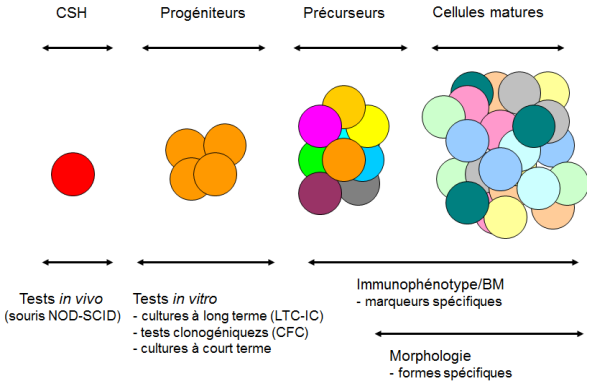
We have two possible pathways for hematopoiesis:

- MYELOPOIESIS = granulopoiesis + monocytopoiesis + erythropoiesis + thrombocytosis
- LYMPHOPOIESIS = LT + LB + NK



There are several stages of differentiation:

- THE**hematopoietic stem cells (HSC**) do not perform a function but have a capacity self-renewal (maintenance of a pool) and multipotent differentiation.
- THE**progenitors**are no longer stem cells, but retain a certain pluripotent character: can for example produce erythroblasts OR platelets; granules OR lymphocytes.



- THE**precursors**are restricted cells committed to a given lineage (e.g. granular, eryhtroblastic, etc.) but do not have the phenotypic and functional characteristics of the mature cell.
- THE**mature cells**perform a function, but are incapable of multiplying.

How to identify these different types of cells?

- For mature cells and precursors: by their morphological, phenotypic and molecular characteristics. Carry differentiation markers.
- For HSCs and progenitors: have no differentiation markers. We must isolate them, put them in culture and determine a posteriori, depending on the type of cells obtained, which progenitor it was.

2. GENERAL INFORMATION ABOUT LYMPHOPOIESIS

2.1. Hematopoiesis model.

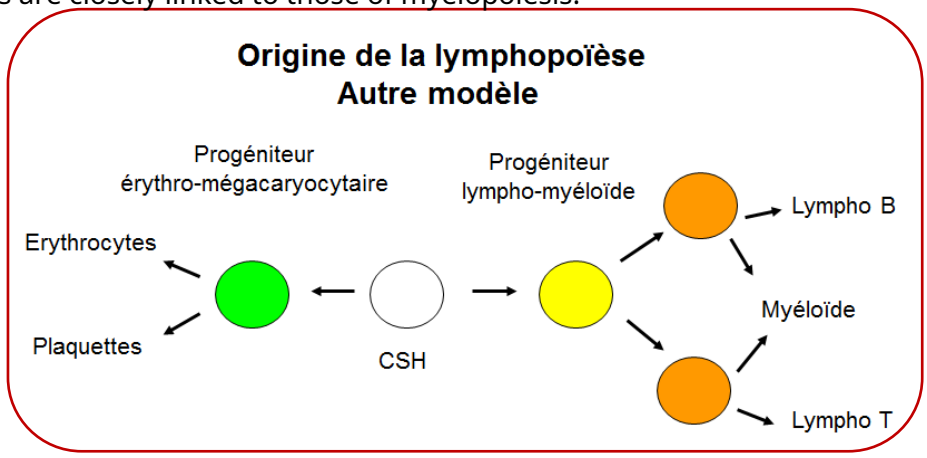
- CLP MODEL CLP = common lymphoid precursor.

The CSH divides quite early into two different lineages, namely the**myeloid lineage and the lymphoid lineage, which remain distinct from each other.**

At the lymphoid level, the CSH then the CLP has the capacity to give B lymphopoiesis in the bone marrow, or to migrate into the thymus to give T lymphopoiesis, or even to give NK lymphopoiesis (not covered in this course).

-CURRENT MODEL

In reality the division is not as drastic as that! The system is more complex. We observe**lympho-myeloid bipotentiality from the CSH**(lympho myeloid progenitor). The pathways of lymphopoiesis are closely linked to those of myelopoiesis.



2.2. Lymphocyte maturation process.

2.2.1.there**primary, early or central lymphopoiesis** .

It is not “driven” by an Ag.

During its maturation process, each lymphocyte creates a unique receptor for an Ag:

- Rc TCR (T receptor)
- Rc BCR (B receptor). The Ac is the excreted form of the BCR, in other words the BCR is a membrane AC.

These receptors are created by recombinational gene mechanics (V, D, J), a source of diversity (see immunology course).

Rque: Gene recombination occurs in two situations that aim for diversity: the development of the lymphocyte repertoire and meiosis (species diversity).

Acquisition of the lymphocyte cell receptor occurs in the primary lymphoid organs:

- **BL = bone marrow**
- **LT = thymus**

TO REMEMBER

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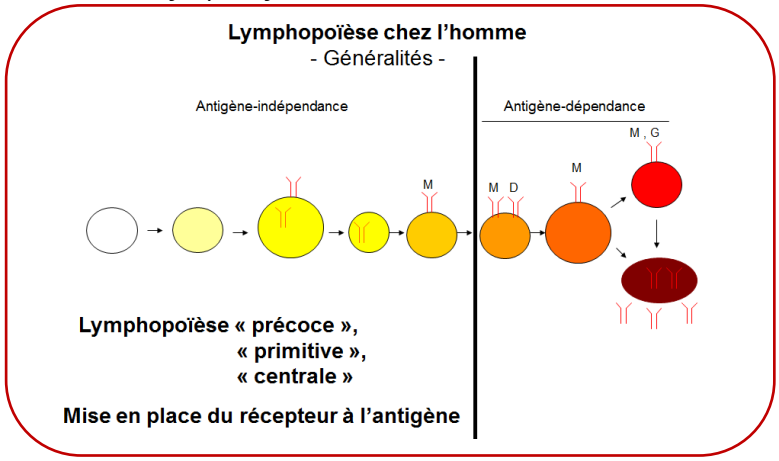
2.2.2. There**secondary, late, peripheral lymphopoiesis**

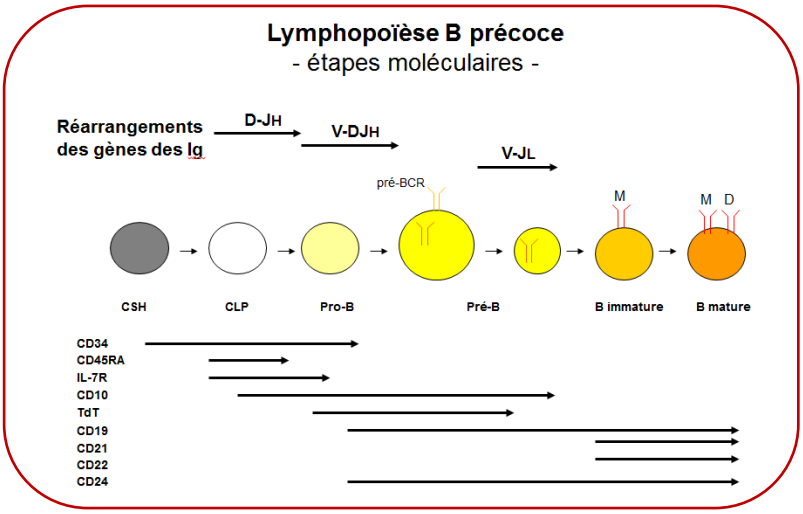
It is Ag dependent, i.e. it occurs by antigenic stimulation. It takes place in the secondary or peripheral lymphoid organs (=the spleen, the lymph node system and the lymphatic system associated with the MALT mucous membranes). It is not a question of neo-lymphopoiesis but of a **amplification of an already formed lymphocyte**. This consists of taking lymphocytes from the repertoire whose receptor recognizes the Ag in question, and**refine**these lymphocytes.



ANATOMIC REMINDER:
Primary lymphoid organs = thymus + bone marrow + liver (embryo only)
Secondary lymphoid organs = spleen + lymphatic network (nodes) + MALT

3. B LYMPHOPOIESIS

It is characterized by different progressive stages which are phenotypic associations.



3.1. Definitions and overview

Lymphopoiesis is the process that allows the production of B lymphocytes. The B lymphocyte is a type of leukocyte (white blood cell) that plays a role in**adaptive immunity**thanks to his**antigen receptor B**(B-cell receptor, or**BCR**or immunoglobulin) and these are the key cells of the**humoral immunity** by their capacity for secretion of**immunoglobulins**(Ig) or**antibody**.

The B lymphocyte is characterized immunophenotypically by the expression of specific antigens such as CD19, CD22, and intracytoplasmically CD79a and CD79b.

B lymphopoiesis is a multistep, highly regulated process that occurs throughout life, first in the fetal liver and then in the bone marrow.

B lymphoid differentiation**early**,**antigen-independent**, which takes place in the**bone marrow** (subject of this course) results in the production in the periphery (blood and lymphatic circulation) of B cells which express on their surface a B antigen receptor (BCR) or surface immunoglobulin (IgM type).

Each B cell expresses a**BCR (or Ig) of different specificity**, thanks to a**almost infinite repertoire**. Each individual has more than 10^9lymphocytes differing in their BCR.

These cells will then gain the**peripheral lymphoid organs**or secondary (nodes, spleen, etc.) where the stages of**antigen-dependent maturation**, Or**immunopoiesis**, leading to **antibody-secreting cells**(**plasma cells**) or some**memory B cells**(covered in another course). Memory plasma cells and B lymphocytes have a long lifespan of several decades, allowing the maintenance of acquired immunity.

3.2. Early stages of B lymphopoisis: lymphoid commitment and loss of multilineage potential

From the pluripotent hematopoietic stem cell (HSC), a highly regulated process will determine the **differentiation into lymphoid progenitors**, which is accompanied by a**loss of multi-lineage potential**. We usually distinguish different stages of**intermediate progenitors**called**LMPP**(lymphoidprimed multipotent progenitor), then**CLP**(common lymphoid progenitor), before the stage **pro-B** definitively engaged in B lymphoid maturation.
This process of**specification**and D'**commitment to a lineage**is regulated by a combination of**transcription factors**and of**signaling pathways**.

CSH / LMPP / CLP / Pro B.

We will mainly remember:

- The transcription factor**IKZF1**(IKAROS) essential to the emergence of the lymphoid lineage
- E2A and EBF factors, "pioneer" factors allowing the opening of B-specific gene loci
- The transcription factor**PAX5**, "master regulator" which induces transcriptional program B and represses the programs of other lineages
- There**interleukin 7 receptor signaling**, which involves IL7 secreted by the cells of the medullary microenvironment and the receptor (IL7-R) present on the surface of lymphoid progenitors.

3.3. Generation of the BCR or immunoglobulin (Ig) repertoire

From the pro-B stage, B lymphoid differentiation allows the generation of a vast repertoire of B cells expressing**Ig of different specificities**in order to recognize all external antigens.

3.3.1. Reminder on the structure of a surface immunoglobulin or BCR

Surface Ig or BCR is made up of 2 identical heavy chains IgH (L=heavy) and 2 identical light chains IgL (L=light). Each chain contains one (for light chains) or several (for heavy chains) constant (C) or invariable domains, and one variable domain (V). The antigen-specific recognition site consists of the combination of heavy and light chain variable domains. The variable domains contain hypervariable regions called "complementarity-determining region" (CDR) located in the loops at the top of each "arm" of the antibodies. The heavy and light chain CDRs determine the specificity of antibodies and interact with the antigen.

The different immunoglobulin chains are encoded by the IGH loci on chromosome 14 for heavy chains, IGK on chromosome 2 for Kappa-type light chains and IGL on chromosome 22 for Lambda-type light chains. Each locus includes one or a few C segments encoding the constant part of the chain as well as **numerous segments V, D and J**constituting the sequence**encoding the variable string**.

IGH IGL IGK

The variable domains are encoded by a DNA sequence unique to each lymphoid precursor, resulting from somatic recombination of genomic segments called V, D, and J, a process that occurs specifically in lymphoid precursors.

C + VDJ

3.3.2. Principle of V(D)J recombination

In each lymphoid precursor and only in the lymphoid precursors, recombination will occur at the level of the loci encoding the immunoglobulin chains, between:

- one of the segments V
- one of the D segments for the IGH locus (D segments absent from the IGK and IGL loci)
- and one of the segments J

This recombination involves phenomena of cutting and ligation ("re-gluing") of the DNA at specific sites, making it possible to join segments V, D and J. This recombination randomly involves the different segments V, D and J loci, with**multiple different combinations possible**.

Several enzymes are involved in this process, in particular:

- THE**recombinases**, coded by genes**RAG1/2**, which are capable of recognizing the specific sequences at 5' and 3' of segments V, D and J called "Recombination-Signal-Sequence" (**RSS**), allowing them to cut the DNA on either side of the segments
- Artemis which makes it possible to cut the hairpins formed by recombinases
- Terminal Deoxynucleotidyl Transferase (**TdT**), enzyme that adds nucleotides randomly to the ends of DNA
- The Non-Homogous-End-Joining (NHEJ) complex which re-ligates two DNA ends.

The diversity of the immunoglobulin repertoire therefore results in:

- of the**combinatorial diversity**between the different segments V, D and J coding for the variable part of the Ig chains

- of the**junctional diversity**resulting from sequence alterations (random deletion and addition of nucleotides) at the junction between segments

3.4. Regulation of Ig gene recombination during lymphoid differentiation

The process of Ig gene recombination is sequential during lymphoid differentiation and highly regulated.

It starts at the stadium**pro-B**thanks to the expression of the RAG1/2 and TdT genes and first affects the IGH locus, with a recombination between a D and J segment, then between a V segment and the DJ sequence. The resulting VDJ sequence can thus give rise to the expression of a μ-type heavy chain (with the Cμ segment used at this stage).

3.4.1. Role of the pre-BCR, notions of productive rearrangement and allelic exclusion

Stadium**pre-B**which follows is characterized by the expression of a**pre-BCR**formed of the heavy chain μ and an invariable pseudo-light chain formed of VpreB and -5. This leads to the temporary cessation of recombinase activity and a phase of cell proliferation allowing the expansion of the precursor population. This phase is followed by re-expression of RAG1/2 which allows recombination of the IGK locus encoding light chains to be initiated.

During V(D)J recombination, the randomly formed junctional sequence may not respect the reading frame and result in a premature stop codon, giving rise to a nonfunctional chain. We then say that the rearrangement is non-productive. The expression of a pre-BCR is only possible in the event of **productive rearrangement**and therefore plays the role of**biological detector (or quality control)**. Only lymphoid precursors expressing a functional μ chain will be able to receive the cell survival and proliferation signal mediated by the pre-BCR (RAF and MAP kinase signaling pathways). This signal, by putting an end to IGH recombinations, also participates in the phenomenon**allelic exclusion**, corresponding to the fact that a B cell can only express one of the two alleles of a locus encoding Ig chains. It is therefore “mono-specific” and the other alleles are non-rearranged or non-productive.

3.4.2. BCR expression, positive and negative selection

Following the expression of the pre-BCR, the alleles of the IGK and IGL loci will be successively rearranged (first IGK then IGL) until a productive rearrangement is obtained allowing the expression of a functional light chain . Isotypic exclusion refers to the fact that a B cell can express either a Kappa or a Lambda chain but not both.

The B cell then reaches the immature B cell stage capable of expressing a complete BCR or surface IgM. Associated with other molecules (CD79a, CD79b, CD19, CD21, etc.), it will mediate a survival signal, this is the phenomenon of**positive selection**and precursors not expressing a functional BCR will be eliminated.

There is also a phenomenon of**negative selection**which occurs when the B cell expresses a BCR recognizing a self-antigen. This**Self-reactive BCR**then transmits a signal**pro-apoptotic**, making it possible to eliminate B cells likely to react against self-antigens (immune tolerance). Alternatively, a self-reactive B cell can escape death by re-initiating the rearrangement of an IGL allele to produce a new light chain and thus modify the specificity of the BCR (receptor editing process).

Thus, the immature B cell expressing a functional surface immunoglobulin will become a naive mature B cell, leave the bone marrow and circulate in the periphery to possibly encounter a potential external antigen and participate in adaptive immunity.

3.4. Examples of disruptions in B lymphoid differentiation in human pathology

3.4.1. Constitutional pathologies linked to V(D)J recombination defects: deficits severe combined immune system (SCID)

These diseases are linked to constitutional (=germline=innate) genetic mutations affecting genes involved in the V(D)J recombination process, such as the RAG1/2 and Artemis genes. They result in B and T lymphopenia which results in severe and recurrent infections occurring from a very young age (“baby bubbles”).

3.4.2. Acquired pathologies: B acute lymphoblastic leukemia (B-ALL)

The V(D)J recombination process is an "error-prone" process that can inappropriately affect genome sequences that are not GI loci. These sequences generally present sites called RSS-like which are recognized by recombinases. Such inappropriate recombination events can generate genomic abnormalities such as chromosomal translocations or deletions, which can activate a proto-oncogene or inactivate a tumor suppressor gene.

RAG-mediated deletions often involve genes encoding key B lymphopoiesis transcription factors such as IKZF1 or PAX5, which act as a tumor suppressor gene or "gatekeeper" in this context. This results in a blockage of differentiation and proliferation of B lymphoid precursors, which become leukemic lymphoblasts.

4. LYMPHOPOIESIS T

It takes place exclusively in thethymus.

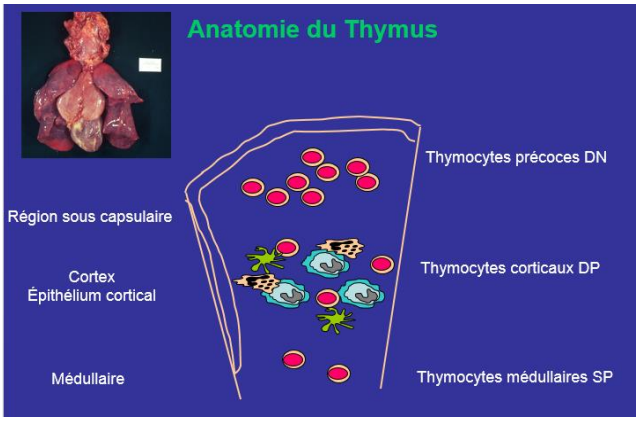
Current question in research: the most multipotent thymic progenitor ≠ CSH (in fact: cannot perform erythropoiesis and megakaryopoiesis, however is not completely restricted; it can give rise to dendritic cells, lymphocytes and do granulopoiesis). HSCs do not migrate into the thymus. We do not know how colonization of the thymus occurs.

4.1. Anatomy of the thymus.

This is a large organ in the child who will undergo a**involution**. It will become an adipose border and no longer be detectable, anatomically, at puberty. Unlike B lymphopoiesis, which continues throughout life, the LT repertoire is therefore made BEFORE puberty.

From the periphery towards the depth, several regions:

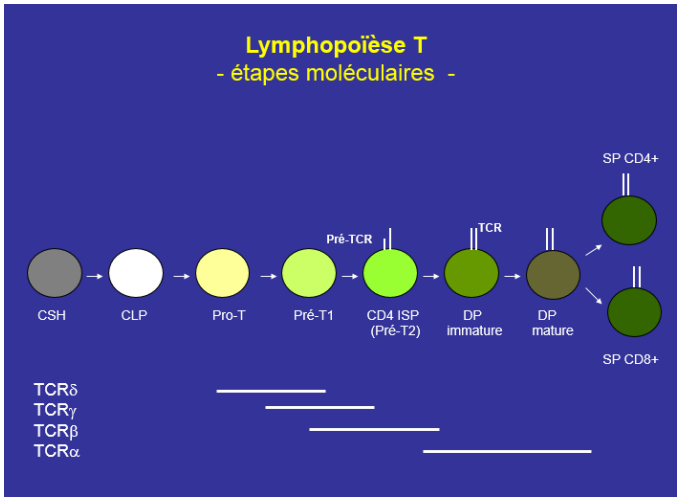
- Regionsubcapsular : area where the most immature thymocytes are found (double negative DN)
- Regioncortical : zone where the thymocytes migrate towards the depth and where the **establishment of the T receptor for Ag**.
- Regionmedullary : place where the most mature thymocytes are found SP=single positive (or CD4+ or CD8+).



4.2. Molecular stages of T lymphopoiesis.

More complicated than B lymphopoiesis because it is not linear: includes several branches:

- Training of the TCR, which can be:
  - A δγ heterodimer
  - An αβ heterodimer
- Within each category (δγ or αβ), choice of becoming:
  - CD4+
  - CD8+



We still have similarities with B lymphopoiesis: we start from an immature cell to form a mature cell; same step of amplification of cells having succeeded in making a productive rearrangement of the β chain of the TCR (=β selection).

β selection occurs in the cortex of the thymus with a CD1a+ (cortical marker) and CD4/CD8 double positive phenotype (i.e. the thymocytes express both CD4 and CD8).

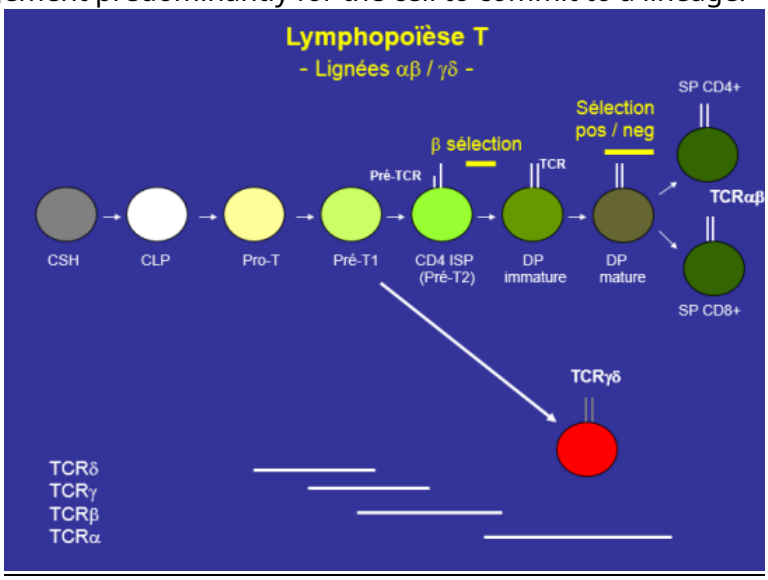
TCR redesigns :First δ rearrangement, then very quickly a γ, later a β and finally an α which occurs after the selection of the cells having made a productive β. The β rearrangement allows the formation of the preTCR which, like the preBCR, gives a β selection signal allowing amplification in the cortex of "good students"

In the body, we have 50 times more LT αβ than γδ.

Differentiation towards the γδ lineage is very early: these thymocytes do not undergo β rearrangement (this does not concern them since they do not have a β chain!).

3 models to explain the rapid bifurcation:

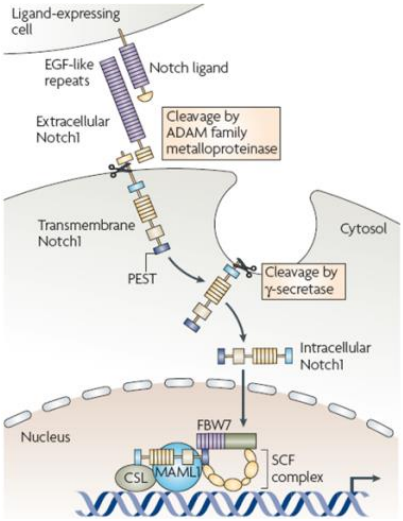
- Stochastic model (probably false): The γδ rearrangement occurs before the αβ, so if we fail to make a productive γδ, we have an αβ BY DEFAULT
- Competitive model: αβ lineage favored because it only requires the β rearrangement. In the γδ lineage, the γ rearrangement and the δ rearrangement must be productive at the same time, whereas in the αβ lineage, a successful β is sufficient to benefit from β selection and carry out clonal amplification.
- Pre-commitment model (arguably the truest of the three): there is predetermination even before the rearrangements, with transcription factors directing this or that rearrangement predominantly for the cell to commit to a lineage.



4.3. Regulation of T lymphopoiesis.

Notch1++++which is a transmembrane receptor, and also has a role as a transcription factor. It is essential.

The membrane receptor is in 2 pieces: one**extra cellular part**→ fixation of the Notch ligand (contact with presenting cell necessary) which leads to the cleavage of the EC part, which is endocytosed. A second series of cleavages frees**the IC part**of Notch which becomes a TF migrating into the nucleus to regulate gene expression.



Signalisation physiologique de NOTCH1

FBXW7 : Composant de l'E3 ubiquitine ligase, liaison au domaine PEST de NOTCH1 pour ubiquitination et dégradation par la voie du protéasome

4.4. LAL T.

As seen previously, this is**malignant and clonal proliferations, with blockage of differentiation, from T precursors.**They are more tumorous with mediastinal masses. Predominantly male.

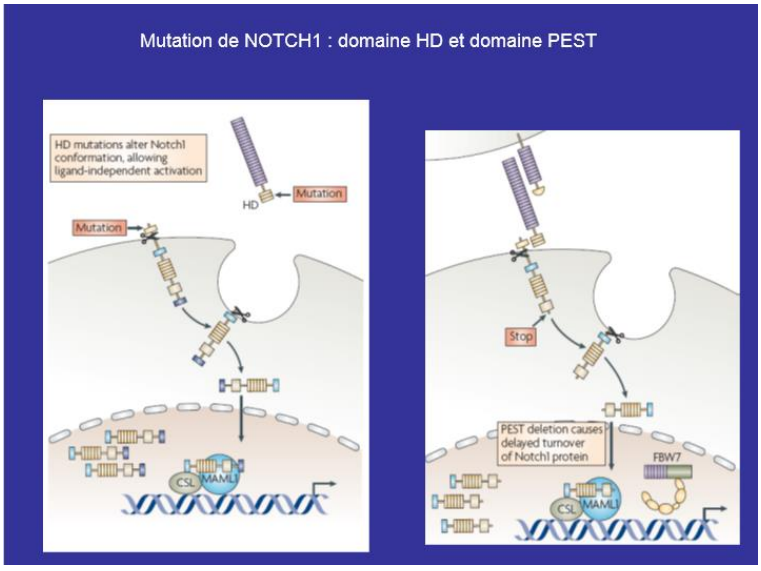
There are three categories of T-ALL depending on the stage of thymic differentiation:

- LYL (double negative stage – these are the subcapsullar thymocytes)
- HOX11 (early cortical stage)
- TAL1 (late cortical stage)

In more than 70% of cases of T ALL, we have**activating mutations**of NOTCH.

1) Mutation concerning the EC part (HD domain): the protein self-cleaves without fixing the ligand.

2) Truncating mutation of PEST which is a negative regulatory domain -> increase in the half-life of the IC part of Notch which can no longer be ubiquitinated and therefore degraded.



Prognosis of T-ALL depends on the presence or absence of these Notch mutations:

- With Notch pathway mutation: better prognosis
- Without Notch pathway mutation: poorer prognosis

We can stratify patients according to their type of T-ALL (i.e. their mutational profile for the Notch pathway), and therefore provide personalized treatment with specific chemotherapy.

It's here**molecular stratification of pathologies.**

5. Immunodeficiencies.

5.1. At the level of B lymphopoiesis; Bruton's disease.

This is deregulation by default.

The example here is the disease of**Bruton**:we have a deficiency of Bruton tyrosine kinase (Btk) → blockage of maturation at the preB stage.

The other name of this pathology is**Sex-linked agammaglobulinemia**because the gene which codes for Btk is located on the

5.2. At the level of T lymphopoiesis; DiGeorge syndrome.

This syndrome corresponds to an embryopathy which affects the third and fourth branchial arches. We have not **no establishment of the thymus (thymic agenesis)**and therefore no T lymphocytes. This agenesis will lead to hypocalcemia and frequent malformations of the vessels of the heart.

Conclusion :

Primary lymphopoiesis = highly regulated process allowing the establishment of immunocompetent cells from immature precursors
Dysregulation by default or excess causes rare but serious human diseases.