Molecular & cellular aspect of lymphocyte maturation – T cells

Comparison with B-cells development:

- Similarities: **orderly** and **stepwise** rearrangement, **testing** rearrangement AND eventual assembly of the **heterodimeric** receptor
- Differences: **2 distinct sets** of TCR encoded by different genes – **$\alpha\beta$TCR** & **$\gamma\delta$TCR** AND more **diverse subsets** of TCR – **helpers** & **killers**

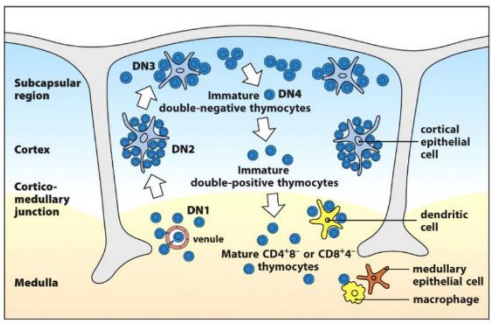
Thymus:

- **Develop fully** at **birth**, **involutes** (the shrinkage of an organ in old age or when inactive e.g. of the uterus after childbirth) during **puberty**
- **Thymic stroma cells** are crucial for **function** of the **thymus** >>> production of **new T-cells** only occur for the **first 6 years** of life BUT some production is kept **throughout life**
- '**Graveyard** of T-cells': only about **5% T-cells** would **survive** >>> lots of dead T-cells in the thymus
- **DiGeogre** syndrome: thymic **aplasia** >>> failure of organ development >>> **NO T-cells**

Thymocytes development:

- Thymocytes enter thymus via **high endothelial venules** (HEV) AND interaction with thymic **stroma** cells initiating T-cell **fate decision** & **proliferation**
- Three **principle** fates:
  1. **$\alpha\beta$TCR**: conventional T-cells, further differentiate into CD4+ or 8+ T-cells
  2. **$\gamma\delta$TCR**: produced **earlier** in **embryonic development** than **conventional** T-cells, do NOT have **naïve** state (**straight** developed into **activated phenotype**), do NOT undergo **positive** or **negative** selection, lack **CD4** & **8**, AND primarily located at **epithelial** & **mucosal** sites >>> respond to **native antigen** probably
  3. **Invariant NKT cells**: use **alpha** & **beta** chains >>> express TCRs BUT do NOT respond to **proteins** >>> respond to **lipids** that must be processed by cells presented in the context of antigen presenting molecule – **CD1** (not MHC)
- Additional fate:
  1. Mucosal Associated Invariant T Cells (**MAIT cells**): they respond to **bacterially-produced vitamin B metabolites**

DN to DP differentiation:



DN (equivalent to **pro-B cells**) = double negative, no expression of **CD4** & **8 receptors**

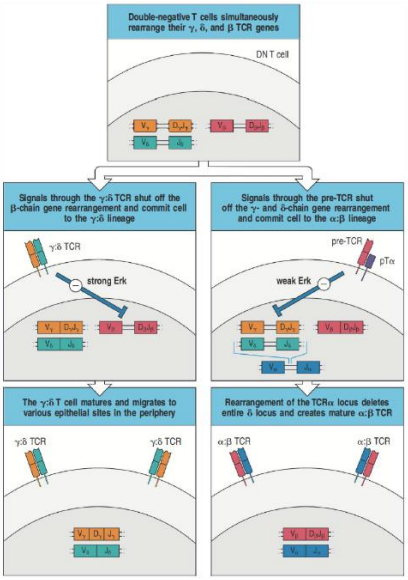
DN1: TCR genes still in **germline configuration** >>> DN1 arrive **thymus** within a **venule** >>> enter thymus at the **corticomedullary junction** >>> as they move around **cortex**, they change their phenotype (**DN2, 3, 4**)

DN2: marked by becoming **responsive** to **IL-2** (**CD25**); **β** chain **rearrangement** commences ($D\boldsymbol{\beta}$ to $J\boldsymbol{\beta}$)

DN3: **rearrangement** continues with $V\boldsymbol{\beta}$ to $DJ\boldsymbol{\beta}$; **pre-TCR** testing determines whether the cells express both **CD4** & **CD8**

DP (equivalent to **pre-B cells**): enters **medulla** and starts **α** chain **rearrangement**; a **single** **β** chain can associate with **many** **different** **α** chains

TCR rearrangement:



In both T- / B- cells' development, the **default response of any cell undergoing rearrangement** is **death** >>> the ONLY way these cells are preserved is that they are **actively** from the **pre-TCR/BCR signals** >>> **95%** T-cells die BECAUSE it is hard to make **proper** $\boldsymbol{\beta}$ chain

With TCR rearrangement, DN cells simultaneously rearrange $\boldsymbol{\beta}$, $\boldsymbol{\gamma}$ and $\boldsymbol{\delta}$ chains:

- If $\boldsymbol{\beta}$ chain rearrangement is <u>successful</u>, the cells form a **pre-TCR** ($\boldsymbol{\beta}$ chain + **pT**$\boldsymbol{\alpha}$ as surrogate $\boldsymbol{\alpha}$ chain) >>> **pre-TCR dimerization** <u>induces</u> **proliferation** >>> then, 1. **Shuts down RAG**, 2. **Induces allelic exclusion** & 3. **Excision of** $\boldsymbol{\gamma\delta}$ **gene** >>> subsequently, $\boldsymbol{\alpha}$ chain rearrangement <u>begins</u> >>> become $\boldsymbol{\alpha\beta}$ **T-cell**

Note: similar to B-cells, we have 2 attempts with $\boldsymbol{\alpha}$ chain rearrangement (2 alleles of the 1 locus)

- If $\boldsymbol{\beta}$ chain rearrangement is <u>unsuccessful</u>, the cell still can form $\boldsymbol{\gamma\delta}$ **T-cell**

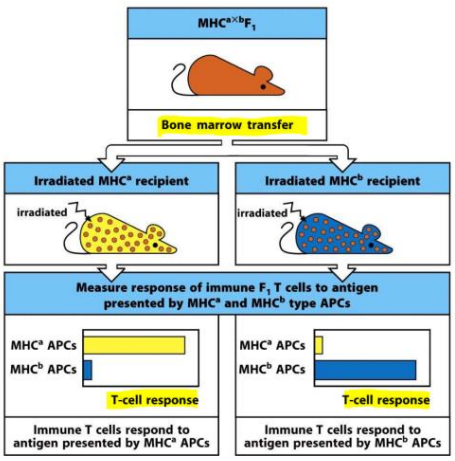
T-cell selection:

B-cells, the **higher affinity** that they have with **endogenous peptide**, the **more likely** it will be **deleted**

T-cells,

- It must have **certain** ability to recognise **endogenous peptide** – MHC molecules >>> therefore, 2 things must be tested for T-cells: 1. **Whether it can bind to MHC** 2. **How strongly it binds to MHC**
- **MHC** molecule will be **UNSTABLE** if there is **NO** antigen presented within it >>> therefore, T-cells will never encounter a MHC molecule that is **EMPTY**
- Now, T-cells need to <u>determine</u> if the peptide **within MHC** is specific (self-reacting) to its TCR >>> if it is a **cognate** peptide (specific to for that TCR), its binding **affinity** NEEDS to increase for **activation**
- **Overall**, after being able to bind to MHC, it is the measurement taken by T-cells to <u>determine</u> if the antigen is recognised or NOT, through detecting the **binding affinity**

<u>Positive selection</u>: test binding to MHC



Irradiate mouse to <u>eliminate</u> their **highly proliferating cells** >>> ALL the **immune cells** are affected WHEREAS **thymic stroma cells** (**non-immune**) are **preserved** due to their **slowly proliferating property**

Then, we use **bone marrow transplantation** to put **immune stem cells** into the mouse (**MCH a + b** bone marrow into **1 recipient**) >>> NOW, their immune system expresses BOTH MHC molecules while their **stroma** ONLY express **a** OR **b**

Findings: T-cells ONLY <u>develop</u> responses to the MHC molecule types of the **stroma type** >>> it tells us that even the bone marrow has the capacity to respond to both **a** & **b**, its **non-immune** cells – **stroma** determines which type of T-cells **survive** (NO MHC signal >>> T-cell death)

Negative selection: test binding to self-antigen

Positive selection ensures that **thymocytes** can **recognize and bind to MHC molecules** AND eventually **determines which subset they belong to** (recall, interaction with thymic **stroma** cells initiating T-cell **fate decision**)

Negative selection: test positively selected thymocyte for **reactivity** >>> in order to avoid immunity, thymocytes binding to self-antigen in a **strong manner** has to be eliminated >>> negative selection is NOT on **thymic stroma cells** BUT on antigen presenting cells (APCs)

General principle:

- Recognition of specific peptide & MHC complex in the **developmental environment** (bone marrow + thymus) leads to **deletion**
- Recognition of specific peptide & MHC complex in the **periphery** leads to **activation**

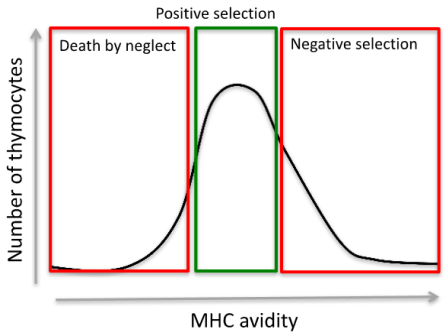
Representation of peripheral self:

- **AIRE**: autoimmune **regulator** >>> it is **transcription factor** that can induce expression of all sorts molecules to provide **periphery self-molecules** in the **thymus**
- **APECED**: autoimmune polyendocrine syndrome, **mutation** on **AIRE** >>> NO **negative selection** within the thymus >>> **multiple** autoimmune diseases
- **IPEX**: another autoimmune disease – lack the ability to make **FOXP3 positive CD4+ T-cells** (**Treg**) >>> with **Treg**, they undergo the **same selection process** BUT **selected in a different way**
- Patients with **APECED** may survive for **decades** WHEREAS patients with **IPEX** would not >>> it suggests that the **periphery tolerance** is MUCH MORE **important** than **central tolerance**

Role of co-receptor CD4/8 in negative selection:

- These **co-receptors** bind to MHC class 1 & 2 molecules >>> increase the **affinity** of TCR interacting with MHC molecules
- If the T-cells do NOT express CD4/8 in context of binding MHC class 1/2 molecules, the **negative selection** is MUCH **less effective**
- Consequently, some self-reactive T-cells will NOT be **negatively selected** AS MUCH AS the **presence** of CD4/8 co-receptors

Treg formation:



Once the T-cells have generated a **proper** β chain >>> it will survive and undergo **positive** & **negative** selection

In between positive & negative selections, there are T-cells **stronger than the positive selection** BUT **not strong enough to trigger negative selection** >>> **Treg** cells are derived from them

Periphery tolerance: If **Treg** encounters antigen in the **periphery**, they secrete **cytokines** (**IL10** & **TGF-beta**) >>> ensure all the antigens that have NOT been covered by **AIRE** under control

Recall, **periphery tolerance** ALSO provides **insurance** to the **retention** of **self-reactive B cells** that may bind to **pathogens** having **similar epitope** to **self-antigen**