T-cell Maturation

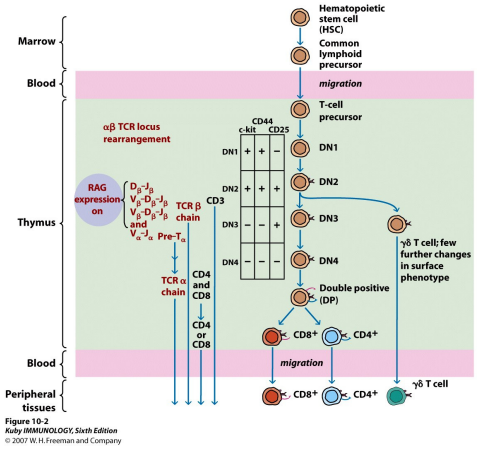
What allows T cell maturation?

- Direct contact with thymic epithelial cells
- Influence of thymic hormones
- Growth factors (cytokines, CSF)

T cell maturation

T cell progenitor → DN (Subcapsular zone) → DP (Cortex) → SP (Medulla) → 2ry lymphoid organs

THYMUS



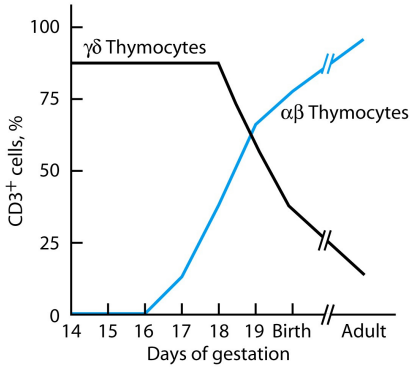
The earliest T cell precursors in the thymus:

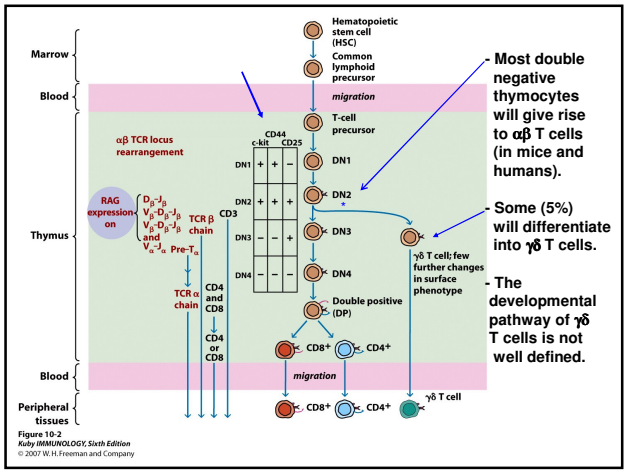
- Express Thy-1 (mice)
- Have not yet rearranged TCR loci
- Do NOT express CD4 or CD8
- Do not express CD3
- Are called "double negatives"

MARKERS:

- C-KIT- Receptor for Stem Cell Growth Factor
- CD44 - Adh. Molecule. Homing to thymus
- CD25 - Alpha chain of IL-2 receptor

Time Course of Appearance in Thymus

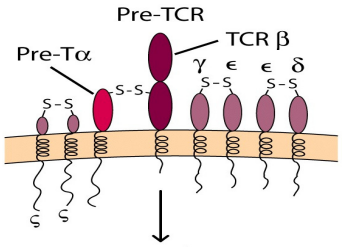




- Most double negative thymocytes will give rise to αβ T cells (in mice and humans).
- Some (5%) will differentiate into γδ T cells.
- The developmental pathway of γδ T cells is not well defined.

- CD3 expression first appear between DN2 to DN3
- A small fraction of DN2 mature into γδ TCR while most cells proceed become αβ TCR
- Double negative thymocytes (DN3 stage) undergo β chain locus re-arrangement.
- The newly formed β chain combines with the Pre-Tα (surrogate chain) and CD3 to form the Pre-T cell receptor (Pre-TCR).

Why is Pre-TCR important?



1) Productive TCR β chain re-arrangement
2) Signals for proliferation (similar β chain) and maturation
3) Suppresses further β chain re-arrangement (allelic exclusion)
4) Signals for TCR α chain re-arrangement
4) Induces development of CD4+8+ (double positive) stage

- After β chain re-arrangement is completed the DN3 cells progress to DN4.
- Both CD4 and CD8 are expressed = now cells are double positive (DP) cells.
- Expression of CD4 and CD8 initiates rearrangement of the α- chain locus in these double positive cells.

- Good: clones with similar β chain but potentially different α- chain locus rearrangement occurs.

- If a productive rearrangement is made, an α/β TCR is expressed on the cell surface.
- Cells undergo positive and negative selection.
- Those that fail either selection undergo apoptosis.
- Those that pass the selection step lose EITHER CD4 or CD8 becoming "single positives"
- These mature single positive cells leave the thymus.

Positive and Negative selection of T cells: GOAL—to recognize foreign Ag combined with self MHC molecules!!!

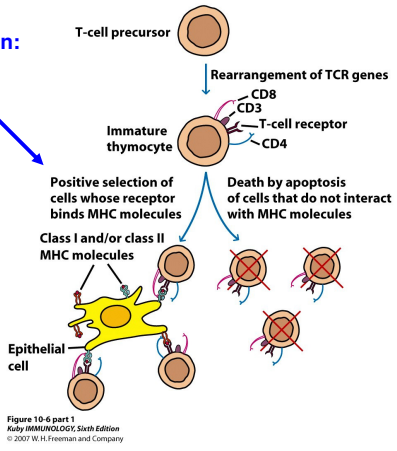
- **Positive selection:** occurs in the cortex and allows only those T cells that are able to bind to self-MHC molecules in the thymus to mature

Positive selection results in MHC restriction.

- **Negative selection:** occurs in the medulla and removes T cells whose TCR strongly recognize (high affinity) self-MHC (with self-antigen). Die by apoptosis within the thymus.

Negative selection results in self-tolerance (to some extent).

Positive selection:



Negative selection:

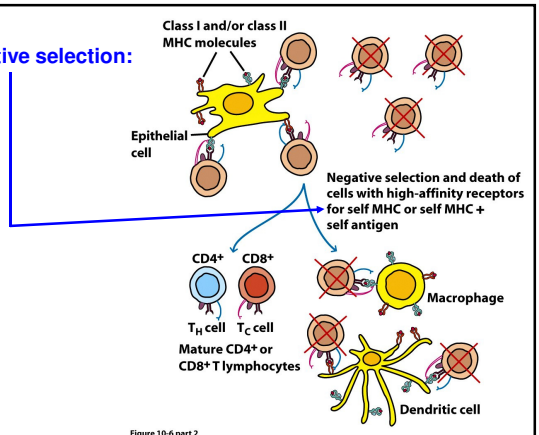
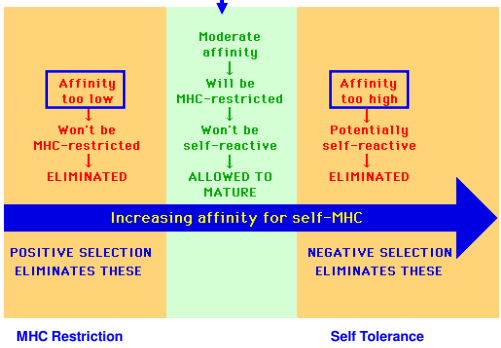
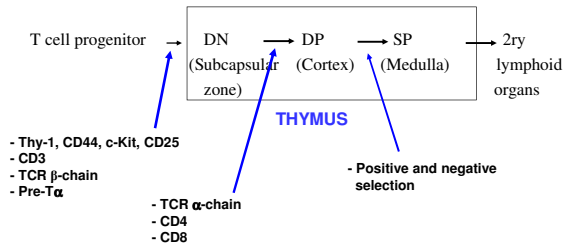


Figure 10-6 part 2
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H.Freeman and Company

Summary:



T cell maturation



Cell distribution in Thymus

TABLE 10-1 Effect of class I or II MHC deficiency on thymocyte populations*

Cell type	Control mice	KNOCKOUT MICE: Class I deficient	KNOCKOUT MICE: Class II deficient
$CD4^-CD8^-$	+	+	+
$CD4^+CD8^+$	+	+	+
$CD4^+$	+	+	−
$CD8^+$	+	−	+

*Plus sign indicates normal distribution of indicated cell types in thymus. Minus sign indicates absence of cell type.

Table 10-1
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H.Freeman and Company

Model to Explain CD4/CD8 Single Positive Cells

INSTRUCTIVE MODEL

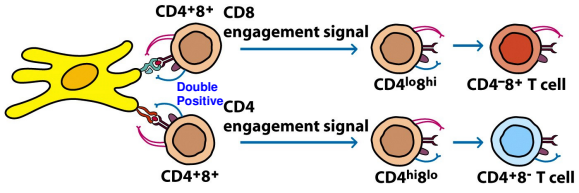


Figure 10-10 part 1
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H.Freeman and Company

- Multiple interactions between TCR, CD8 or CD4 with MHC-I or MHC-II will deliver the "right signal" (strength?) and instructs the cell to differentiate.

Model to Explain CD4/CD8 Single Positive Cells

STOCHASTIC MODEL

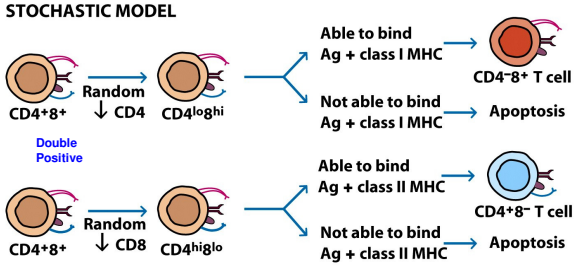


Figure 10-10 part 2
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H.Freeman and Company

- Expression of CD4 or CD8 is switched off randomly
- Only thymocytes with ON co-receptor and correct peptide+MHC complex will mature

Summary of T cell maturation (αβ T cells only)

- Thymocytes enter the thymus as "double negative" (markers?)
- Induces β-chain rearrangement (apoptosis of cells that fail to rearrange β chain correctly)
- Expression of pre-TCR (surrogate α chain)
- Proliferation of similar β-chain clones with surrogate α-chain
- Expression of CD4 and CD8 (to form "double positive" thymocytes)
- α-chain rearrangement (apoptosis of cells that fail to rearrange α correctly)
- Expression of mature αβ TCR
- Positive and negative selection (death of cells with too low or too high an affinity for self MHC…>99% of thymocytes die within the thymus)
- Loss of either CD4 or CD8
- Migration to periphery of cells that successfully complete these steps

T-cell Activation

Protein Kinases – catalyzes the covalent attachment of a phosphate (P) group to a protein

Aminoacids – phosphorylation occurs in tyrosine, serine or thronine

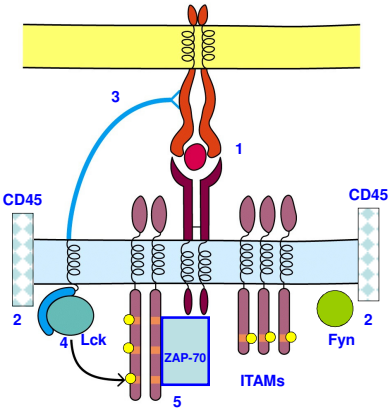
Protein phosphatases – remove phosphate groups from proteins

TABLE 10-2 Time course of gene expression by T_H cells following interaction with antigen

Gene product	Function	Time mRNA expression begins	Location	Ratio of activated to nonactivated cells
IMMEDIATE				
c-Fos	Proto-oncogene; nuclear-binding protein	15 min	Nucleus	>100
c-Jun	Cellular oncogene; transcription factor	15–20 min	Nucleus	?
NFAT	Transcription factor	20 min	Nucleus	50
c-Myc	Cellular oncogene	30 min	Nucleus	20
NF-κB	Transcription factor	30 min	Nucleus	>10
EARLY				
IFN-γ	Cytokine	30 min	Secreted	>100
IL-2	Cytokine	45 min	Secreted	>1000
Insulin receptor	Hormone receptor	1 h	Cell membrane	3
IL-3	Cytokine	1–2 h	Secreted	>100
TGF-β	Cytokine	<2 h	Secreted	>10
IL-2 receptor (p55)	Cytokine receptor	2 h	Cell membrane	>50
TNF-β	Cytokine	1–3 h	Secreted	>100
Cyclin	Cell cycle protein	4–6 h	Cytoplasmic	>10
IL-4	Cytokine	<6 h	Secreted	>100
IL-5	Cytokine	<6 h	Secreted	>100
IL-6	Cytokine	<6 h	Secreted	>100
c-Myb	Proto-oncogene	16 h	Nucleus	100
GM-CSF	Cytokine	20 h	Secreted	?
LATE				
HLA-DR	Class II MHC molecule	3–5 days	Cell membrane	10
VLA-4	Adhesion molecule	4 days	Cell membrane	>100
VLA-1, VLA-2, VLA-3, VLA-5	Adhesion molecules	7–14 days	Cell membrane	>100, ?, ?, ?

SOURCE: Adapted from G. Crabtree, Science 243:357.

Table 10-2
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H.Freeman and Company



Signals through the TCR, CD3 and CD4/8 activate a protein tyrosine kinase (PTK) cascade

- PTK = enzyme that phosphorylates the amino acid tyrosine (Y) in ITAMs

- PHOSPHATASES = enzymes that remove inhibitory phosphates

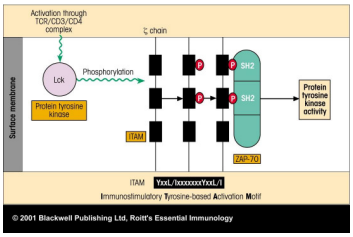


Figure 9.5

Signals through the TCR, CD3 and CD4/8 activate a PTK cascade

- CD4/8 are associated with a cytoplasmic tyrosine kinase enzyme – Lck (lymphocyte kinase)

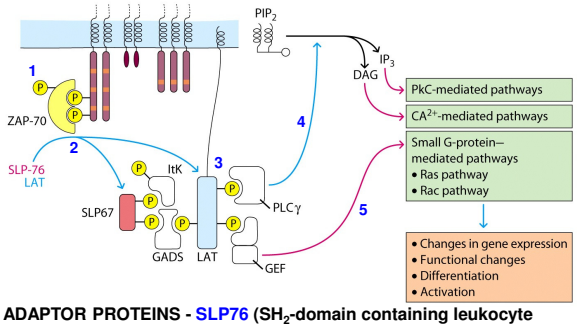
1. TCR-MHC-Peptide activates the phosphathase CD45
2. CD45 removes inhibitory P from Fyn and Lck
3. Activated Lck and Fyn phosphorylate ITAMs in CD3 chains
4. Phosphorylated ITAM motifs on the CD3zz chains become a docking site for the PTK ZAP-70.
5. Binding of CD4 to MHC molecules brings Lck closer to ZAP-70. Lck phosphorylates ZAP-70 to become an active PTK.

- Activation of ZAP-70 initiates a cascade of events that results in phosphorylation of ADAPTOR molecules such as Linker of Activated T cells (LAT) and SLP-76 which phosphorylates and activates phospholypase C (PLCγ1).

- PLCγ1 converts phosphatidyl inositol 4,5-biphosphate (PIP_2) to diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3):

$$PIP_2 \xrightarrow{PLC\gamma1} DAG \text{ and } IP_3$$

- IP_3 triggers the release of calcium from intracellular storage vesicles into the cytosol, thus raising cytoplasmic calcium levels.



ADAPTOR PROTEINS - SLP76 (SH_2-domain containing leukocyte protein of 76 KDa and LAT (Linker of activated T cells) are phosphorylated by ZAP-70.

ADAPTOR PROTEINS: 1) Serve as links for proteins, 2) Promote assembly of membrane proteins

Calcium:

1) Synergizes with DAG to activate protein kinase C (PKC)

2) PKC will activate the transcription factor NF-κB

3) PKC activates IkB kinase (IKK), which phosphorylates IkB, releasing the transcription factor NF-kB – which translocates to the nucleus.

4) Acts together with calmodulin to activate calcineurin (phosphatase)

5) Calcineurin activates the cytoplasmic component of the transcription factor NFAT (NFATc), causing it to translocate to the nucleus (NFATn)

5. DAG stimulates a Ras guanine nucleotide exchange factor (RasGRP) which activates the G protein Ras and the MAP kinase pathway. This pathway activates Erk which then activates Elk. Elk translocates to the nucleus and induces Fos expression. This pathway promotes the AP-1 transcription factor

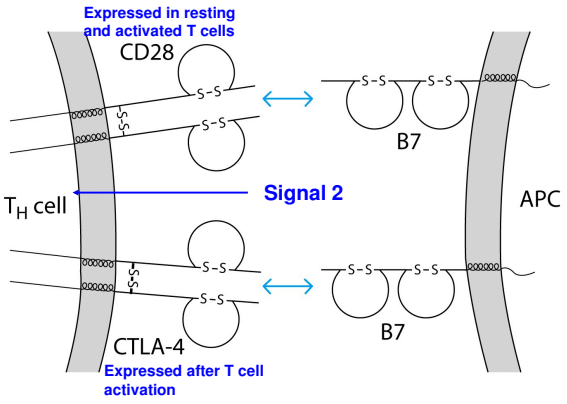
- The expression of >70 genes is increased within 4 hr of T cell activation.

- The potent immunosuppressive drugs cyclosporin and FK506 act by inhibiting the activation of calcineurin → X → NF-AT!

TRANSCRIPTION FACTORS- lead to gene transcription, cell proliferation and differentiation.

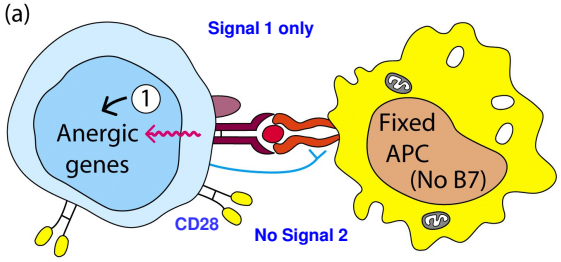
The interaction of CD28 with B7 sends additional activation signals.

- CTLA-4 is not initially expressed, but is expressed after T cell activation.
- CTLA-4 has a higher affinity for B7 than CD28.
- Interaction of CTLA-4 with B7 is thought to down-regulate T cell activation.
- There are actually two related molecules: B7.1 and B7.2
- B7 molecules constitutively expressed in DCs but induced in activated MO and B cells



CLONAL EXPANSION vs CLONAL ANERGY= inability of T cell to respond after TCR-MHC/peptide stimulation

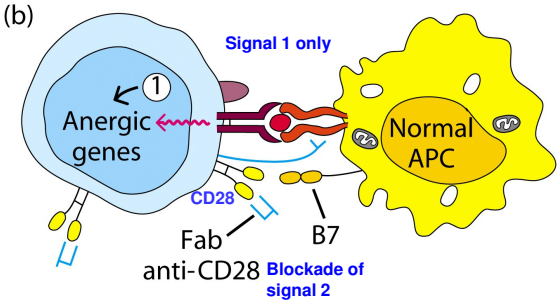
(a)



ANERGY!!!!

CLONAL EXPANSION vs CLONAL ANERGY= inability of T cell to respond after TCR-MHC/peptide stimulation

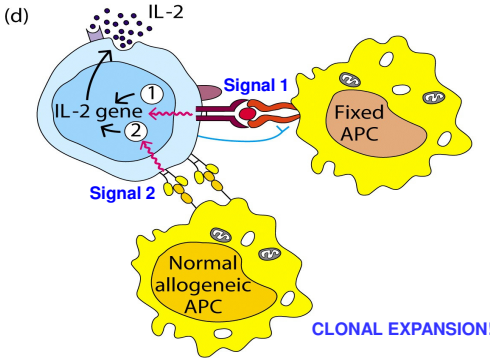
(b)



ANERGY!!!!

CLONAL EXPANSION vs CLONAL ANERGY= inability of T cell to respond after TCR-MHC/peptide stimulation

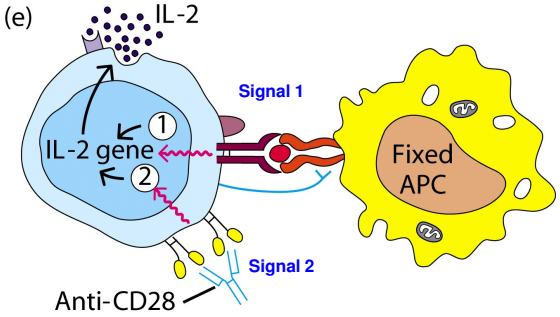
(d)



CLONAL EXPANSION!!!!

CLONAL EXPANSION vs CLONAL ANERGY= inability of T cell to respond after TCR-MHC/peptide stimulation

(e)



CLONAL EXPANSION!!!!

During activation of helper T cells by antigen presenting cells, many cell-cell interactions must occur.

- Signal 1 is the interaction of the TCR with peptide antigen presented on class II MHC (and interaction of CD4 with class II MHC).

- Signal 2 is the interaction is between CD28 (and CTLA-4) on the helper T cell and B7 on the antigen-presenting cell. This provides a co-stimulatory activation signal.

- If T cells receive signal 1 only, they will NOT be activated by antigen. Instead, they will become anergic i.e. they will become refractory to any subsequent activation by antigen.

- If T cells receive signal 1 and signal 2, they will be activated to participate in an immune response to the antigen.

(If T cells receive signal 2 only, nothing happens.)

Superantigens

- Proteins produced by pathogens
- Not processed by antigen presenting cells
- Intact protein binds to the β variable region on TCR of T cells and to MHC class II on antigen presenting cells (APC)
- Large numbers of activated T cells release cytokines having pathological effects
- Endogenous (virus) and exogenous (exotoxins) from Gram (+)

Super Antigens

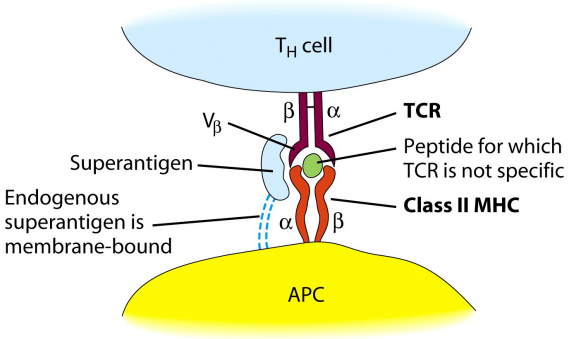


TABLE 10-3 Exogenous superantigens and their V_β specificity

Superantigen	Disease*	V_β SPECIFICITY: Mouse	V_β SPECIFICITY: Human
Staphylococcal enterotoxins			
SEA	Food poisoning	1, 3, 10, 11, 12, 17	nd
SEB	Food poisoning	3, 8.1, 8.2, 8.3	3, 12, 14, 15, 17, 20
SEC1	Food poisoning	7, 8.2, 8.3, 11	12
SEC2	Food poisoning	8.2, 10	12, 13, 14, 15, 17, 20
SEC3	Food poisoning	7, 8.2	5, 12
SED	Food poisoning	3, 7, 8.3, 11, 17	5, 12
SEE	Food poisoning	11, 15, 17	5.1, 6.1–6.3, 8, 18
Toxic-shock-syndrome toxin (TSST1)	Toxic-shock syndrome	15, 16	2
Exfoliative-dermatitis toxin (ExFT)	Scalded-skin syndrome	10, 11, 15	2
Mycoplasma-arthritidis supernatant (MAS)	Arthritis, shock	6, 8.1–8.3	nd
Streptococcal pyrogenic exotoxins (SPE-A, B, C, D)	Rheumatic fever, shock	nd	nd

*Disease results from infection by bacteria that produce the indicated superantigens.

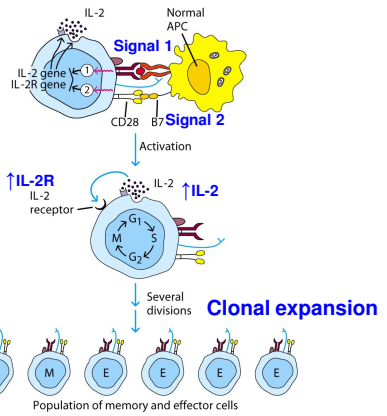
Consequences:

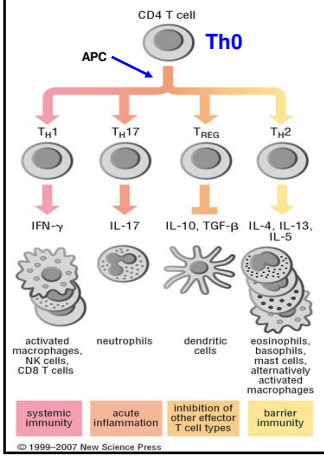
- Because they cross-link the Vβ domain of the TCR with the Vα domain of the MHC-II, this results in non-specific proliferation and activation.
- Over production of Th cytokine leading to systemic toxicity (IFN-γ, TNF-α) and inflammatory mediators.
- Deletion (negative selection) of thymocytes bearing Vβ domains recognized by the super antigen---- beneficial?

T cell differentiation:

- Remember: Naïve T cells continually re-circulate between the blood and lymph system→ search for appropriate antigen
- Once activated (Remember signal 1 and 2) → Primary response where T cells proliferate and differentiate into effector and memory T cells.
- **CD4 effector T cells** can form two subpopulations based on cytokine production: T_H1 subset (IL-2, IFN-γ) and T_H2 subset (IL-4, IL-5, IL-10)
- **T_H1**: associated with cell-mediated functions inflammation (delayed-type hypersensitivity, activation of Macrophages and CD8 T cells); **T_H2**: associated with B-cell activation.

IL-2 critical to T cell activation



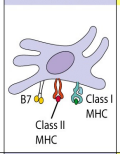
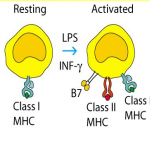
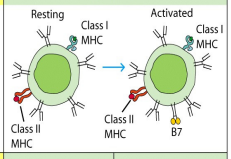


- **Outcome of CD4 T cells**
- **Th_0 will give rise to at least 3 Th populations**
- **Th populations differentiated based on cytokine production**
- **Treg**

Suppressor T cells

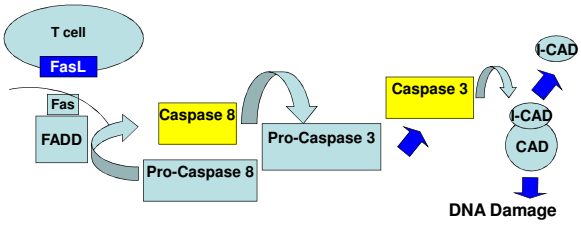
- The old questions revisited!
- First described in the 70's made $CD8^+$ the candidate for Ts cells
- Ts cells are **$CD4^+$** cells with the phenotype **$CD4^+CD25^+$ → $CD4^+CD25+FoxP3^+$.**
- Several potential applications: 1) suppression→ tissue rejection; 2) treatment of allergies or autoimmune diseases; 3) enhance response to vaccines; etc

Why are DC better APCs?

	Dendritic cell	Macrophage		B Lymphocyte	
					
Antigen uptake	Endocytosis phagocytosis (by Langerhans cells)	Phagocytosis	Phagocytosis	Receptor-mediated endocytosis	Receptor-mediated endocytosis
Class II MHC expression	Constitutive (+++)	Inducible (–)	Inducible (++)	Constitutive (++)	Constitutive (+++)
Co-stimulatory activity	Constitutive B7 (+++)	Inducible B7 (–)	Inducible B7 (++)	Inducible B7 (–)	Inducible B7 (++)
T-cell activation	Naive T cells Effector T cells Memory T cells	(–)	Effector T cells Memory T cells	Effector T cells Memory T cells	Naive T cells Effector T cells Memory T cells

Cell death (apoptosis)

- Several apoptotic pathways
- Fas-FasL pathways
- Fas and its ligand FasL are induced upon T cell activation
- Mediated by Caspases

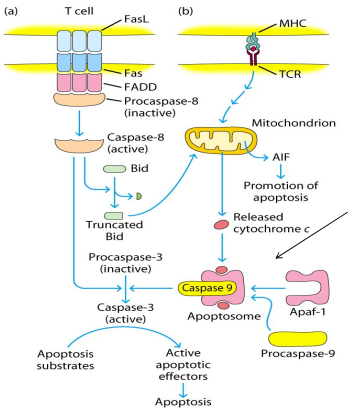


GOAL:

1) **Regulation of T cell numbers,**
2) **Removal of "turned off" T cells**
3) **Deletion of cells with high avidity for MHC**

- Passive cell death – when antigen is no longer available.

Prevent: Bcl-2, Bcl-XL
Promote: Bax, Bak



The End