ANTIGEN PRESENTATION and the MHC

Date: 3/27/13
Reading Assignment: **Janeway's Immunobiology,** 8th Edition, pp. 29-31, 202-209, 210-214, 342-344.

Figures: (Unless otherwise noted) **Janeway's Immunobiology,** 8th Edition, Murphy *et al.*, Garland Publishing.

KEY CONCEPTS AND LEARNING OBJECTIVES

You will be able to describe the role of antigen presenting cells in the development of the immune response with emphasis upon the role of the major histocompatibility complex. You will be able to describe the process of naive lymphocyte activation.

To attain competence for this lecture you will be able to:

a. List the different types of antigen presenting cells.
b. Compare and contrast MHC class I and class II antigen presentation.
c. Identify the signals required to activate T-lymphocytes.
d. Describe the immune synapse.

CONTENT SUMMARY

INTRODUCTION

- The process of displaying antigen by MHC molecules is called antigen presentation.

- Specialized cells displaying antigen and class II MHC molecules are referred to as antigen presenting cells (APCs), even though all nucleated cells express MHC class I molecules and can present antigen via these molecules.

THE MAJOR PROFESSIONAL APCs

APCs take up antigen, either by surface receptors or by phagocytosis and then present it to immunologically competent lymphocytes. MHC class II expression is to a large extent confined to APCs, which are:

- Mononuclear Phagocytes (macrophages)
- Dendritic Cells
- B Lymphocytes.

See **Table I.**

Table I. **Antigen Presenting Cells (APCs).**

Cell Type	Location	Phagocytic	Class II
Mononuclear phagocytes (Macrophages)	Blood, liver, spleen tissues	Yes	Yes
Dendritic cells	Skin, lymphoid tissues	Yes	Yes
B lymphocytes	Lymphoid tissues, sites of immune reactions	No	Yes

- Mononuclear phagocytes when localized in tissues are referred to as macrophages (e.g. macrophages lining the sinusoids of the liver are known as Kupffer cells and those found in the brain are microglial cells. See **Figure 1**.

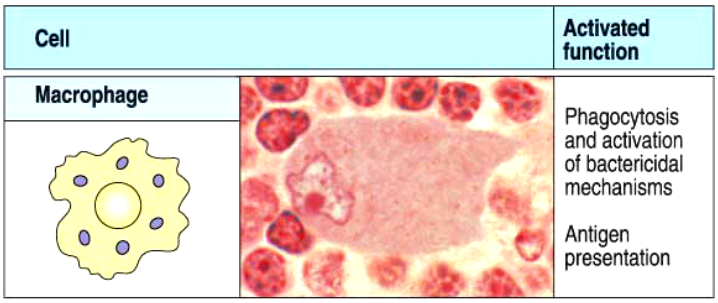


Figure 1. Mononuclear phagocyte. Figure 1.22 in the text.

- Skin APCs are Langerhans' cells, which form a continuous cellular sheet at the junction of the dermis and epidermis. These cells are capable of migrating via the afferent lymphatics into the paracortex of the draining lymph node. Within the paracortex, they interdigitate with T lymphocytes, presenting antigen carried from the skin to immune responsive cells.

- Follicular dendritic cells are found in B lymphocyte areas of lymph nodes and spleen. These cells present antigen to B lymphocytes. See **Figure 2.**

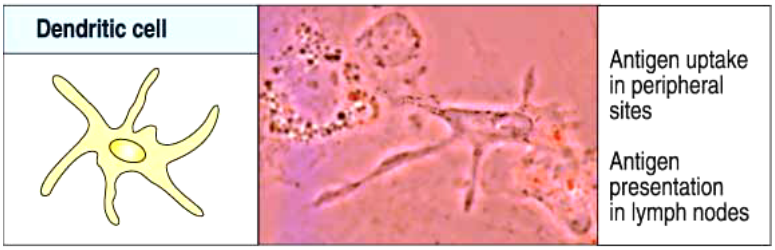


Figure 2. Dendritic cell. Figure 1.22 in the text.

There are two distinct lineages of dendritic cells; **conventional** and **plasmacytoid**. Plasmacytoid dendritic cells produce large quantities of interferon in response to viral infections. Conventional dendritic cells undergo a maturation process depicted in **Figure 3.** Scanning electron micrographs are in the right panels and show maturation from immature at the top to mature dendritic cells at the bottom. In the left panels, green fluorescence depicts MHC class II molecules and red fluorescence depicts lysosomal protein. When the two colors occupy the same cellular locale they appear as yellow. The top panel depicts highly phagocytic immature dendritic cells and the bottom panel depicts non-phagocytic mature dendritic cells that present large quantities of peptide in the context of MHC class II molecules.

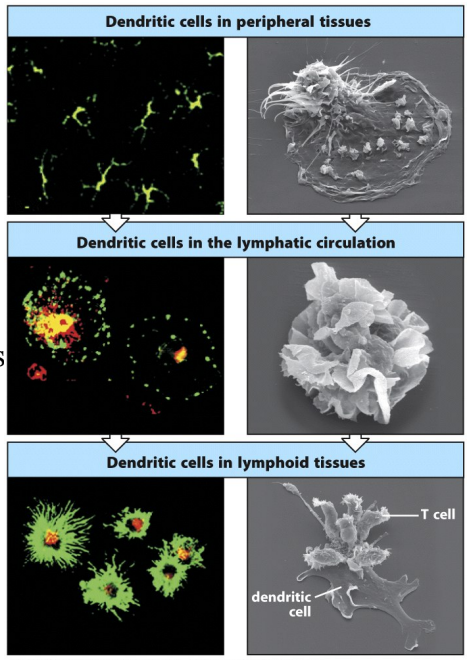


Figure 3. Dendritic cell maturation (Text Figure 9.9).

- B lymphocytes are also rich in surface class II molecules and have been shown to process and present antigen, particularly when the B lymphocyte is immunologically specific for the antigen. B lymphocyte antigen presentation is most important during secondary antibody responses. See **Figure 4**.

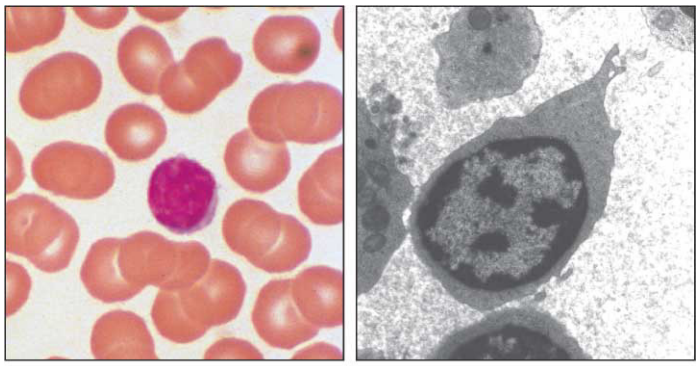


Figure 4. B lymphocyte. Figure 1.7 in the text.

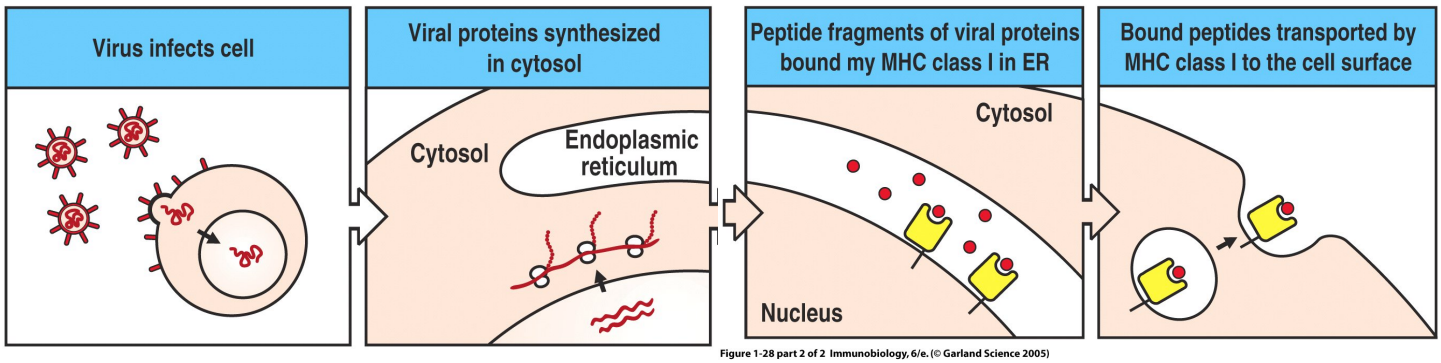
- Macrophages, Langerhans' cells, interdigitating dendritic cells, and B lymphocytes all express MHC class II molecules.

ANTIGEN PROCESSING

- Complex antigens (e.g. cells, proteins) are degraded or processed into small antigenic fragments that are recognizable by T lymphocytes.

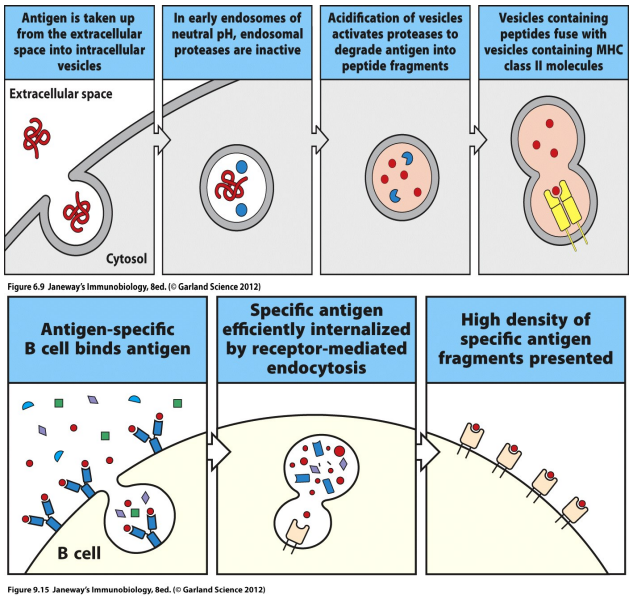
- These fragments are peptides that associate with either MHC class I (see **Figure 5**) or class II molecules (see **Figure 6**).

- The actual association of the antigenic fragments takes place following cytoplasmic production (Class I) of the antigen or alternatively following phagocytosis or endocytosis (Class II) of the antigen.



Text Figure 1.29

Figure 5. Model of MHC Class I antigen presenting pathway.



Text Figures, respectively 6.9 and 9.15

Figure 6. Model of MHC Class II antigen presenting pathways.

MHC CLASS I ANTIGEN PROCESSING

- Antigenic peptides that bind to MHC class I molecules are typically derived from viruses that take over the biosynthetic machinery of the cell, resulting in the production of viral proteins (foreign antigens). These viral proteins are degraded by the host cell's proteasomes [long cylindrical structures, comprised of subunits LMP2 and LMP7 that contain multicatalytic proteases] into small peptide fragments.

- Peptides generated in the cytoplasm are transported into the endoplasmic reticulum by TAP-1 and TAP-2 (Transporters Associated with Antigen Processing-1, -2). See **Figure 7.**

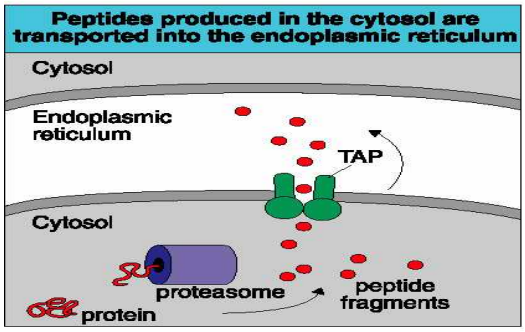


Figure 7. Proteasome degradation of proteins and transport to the endoplasmic reticulum by TAP-1 and TAP-2.

- Newly synthesized MHC lass I molecules assemble in the endoplasmic reticulum with the help of several chaperones (calnexin, Erp57, calreticulin). These MHC class I molecules associate with TAP-1,-2 and when peptides are transported into the endplasmic reticulum they are trimmed by ERAAP (endoplasmic reticulum aminopeptidase associated with antigen processing) and the peptides bind to the MHC molecule, the peptide-MHC complex leaves the endoplasmic reticulum and is transported through the Golgi apparatus to the cell surface. See **Figure 8a and 8b. (**Text Movie 6.1 is not assigned but is well done and illustrative of this process.)

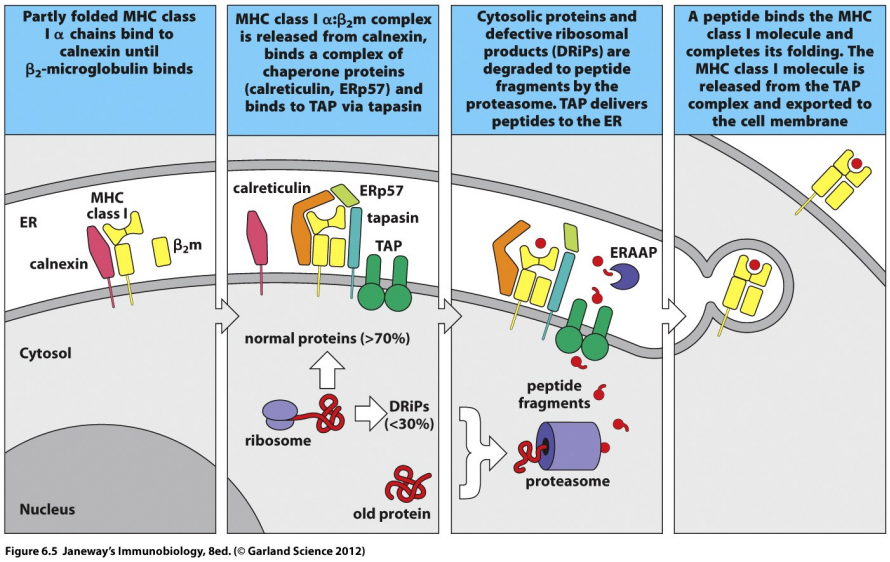


Figure 6.5 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Figure 8a. Peptide loading and transport of MHC Class I molecules to the cell surface.

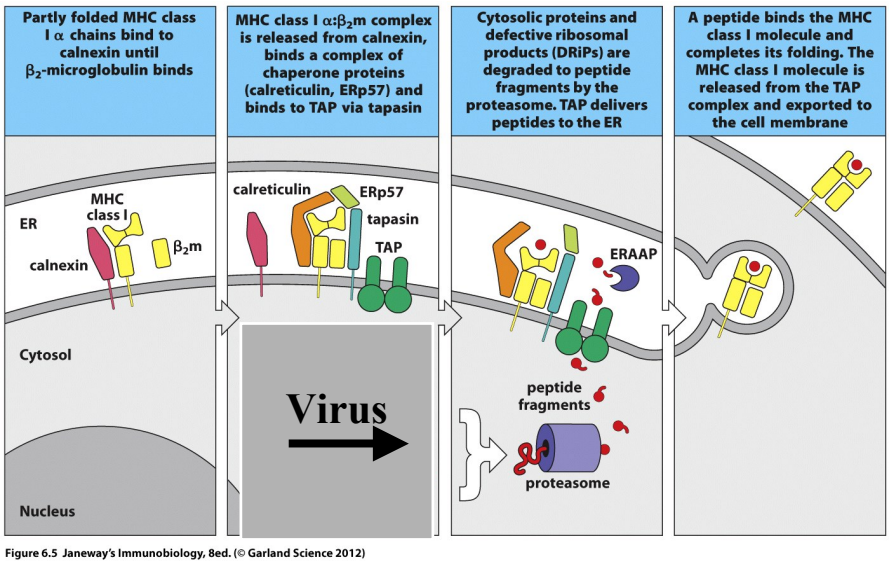
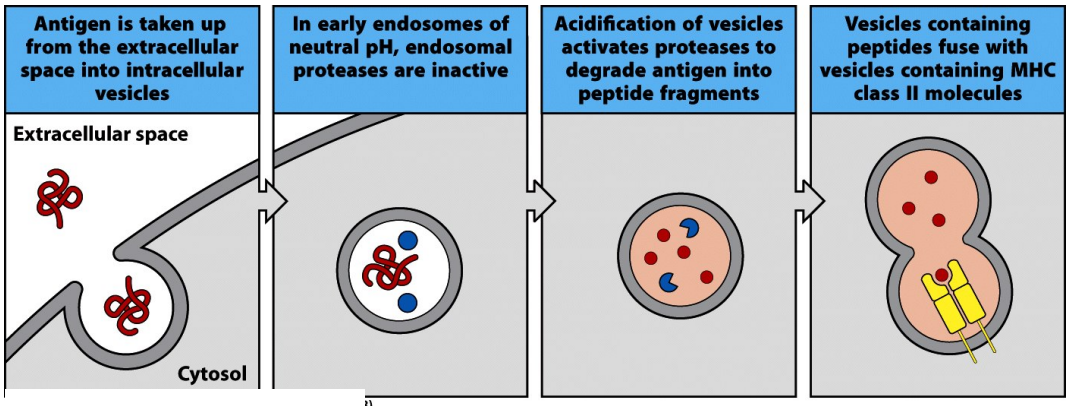


Figure 6.5 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Figure 8b. Peptide loading and transport of MHC Class I molecules to the cell surface.

MHC CLASS II ANTIGEN PROCESSING

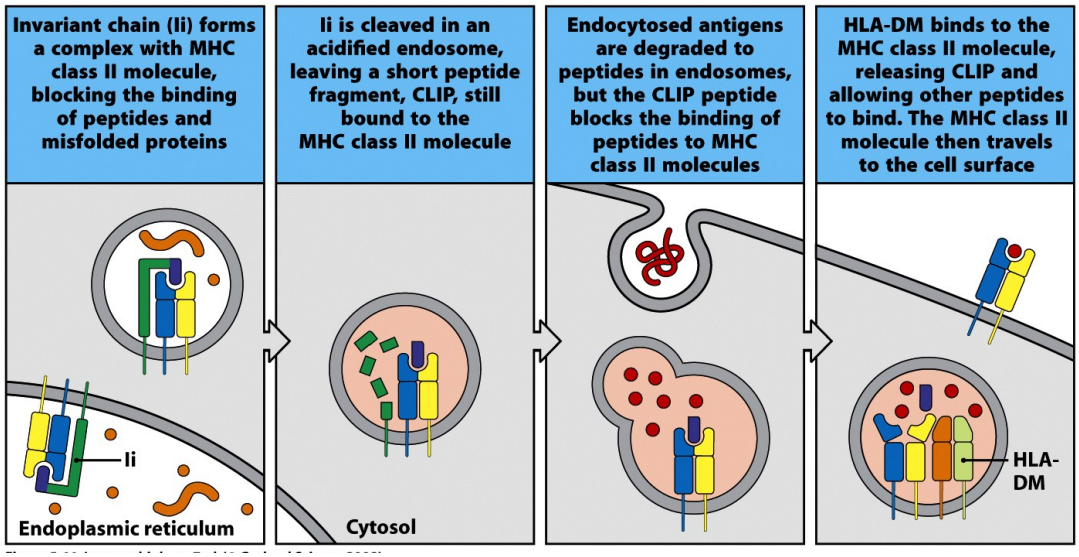
- MHC class II associated peptides are derived from antigens captured and internalized by specialized APCs. These antigens are degraded enzymatically, in endosomes and lysosomes, into peptides that bind MHC class II molecules. See **Figure 9**.



Text Figure 6.9

Figure 9. Peptides that bind to MHC class II molecules are generated in acidified endocytic vesicles.

- Class II MHC molecules are synthesized in the endoplasmic reticulum and are transported to endosomes with an associated protein, invariant chain (Ii), which occupies the peptide binding cleft of the newly synthesized MHC class II molecule. Within the endosome, acidification cleaves Ii leaving a short peptide fragment, CLIP (class II-associated invariant chain peptide), bound to the peptide binding groove of the MHC class II molecule. Once



Text Figure 6.12

Figure 10. Peptide loading and transport of MHC Class II molecules to the cell surface.

such endosomes fuse with a vesicle containing foreign antigen, CLIP is removed by a peptide unloader/loader, DM, which then places foreign peptides in the groove of the MHC class II molecule. (Degradation of Ii increases the mobility of these antigen presenting cells.) The peptide MHC complex then transits to the cell surface See **Figure 10**. **(**Text Movie 6.3 is not assigned but is well done and illustrative of this process.)

The process of peptide loading and transport of MHC class I and class II molecules to the cell surface is summarized in **Figure 11**.

It is worth noting that MHC class I molecules under normal conditions (in the absence of foreign antigen) are loaded with self peptides derived from the normal degradation of self cellular proteins. MHC class II molecules, under normal conditions, are thought to contain only CLIP in their peptide binding groove.

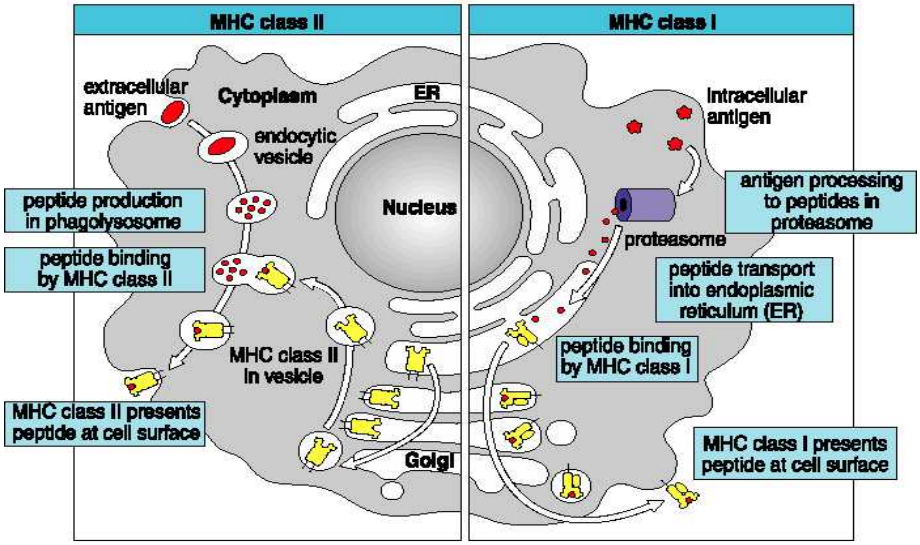


Figure 11. Summary of peptide loading and transport of MHC Class I and II molecules to the cell surface.

MHC CLASS II RESTRICTED PRESENTATION OF ANTIGEN TO T-LYMPHOCYTES

- CD4+T lymphocytes do not recognize free or soluble antigens.
- Rather, they recognize antigen on the surface of APCs in the context of class II molecules.
- The interaction between T lymphocytes and antigen and the class II molecule is highly specific and will result in specific T cell proliferation and differentiation.

- These lymphocytes are said to be genetically restricted by the class II molecule on which the antigenic determinants was first recognized. This is called MHC restriction.
- CD4+ T lymphocytes can either mediate macrophage activation or act as helper cells in antibody responses (by secreting cytokines). See **Figure 12**.

- The subsets of CD4+ T lymphocytes that:
    - Activate macrophages are designated Th1.
    - Induce antibody synthesis are designated Th2.

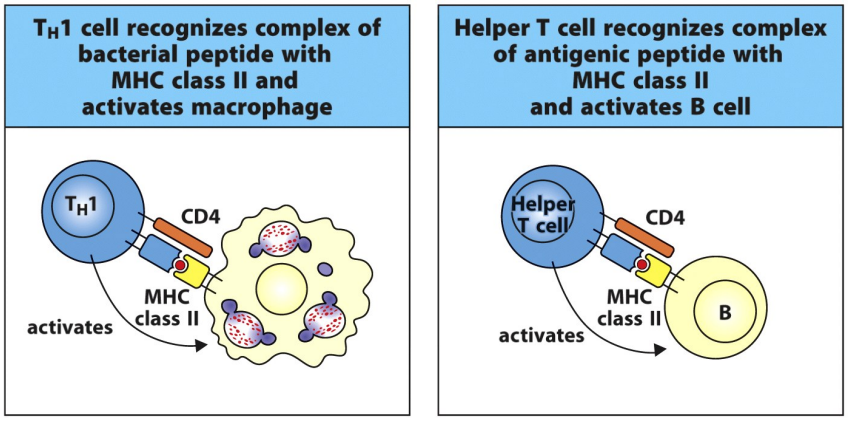


Figure 12. Complex of antigen fragment and class II MHC molecule forms the ligand for the TCR (T cell receptor for antigen).

T LYMPHOCYTE ACTIVATION

ACTIVATION OF NAÏVE T CELLS BY INTERACTION WITH ANTIGEN PRESENTING CELLS

- Resting (immature) APCs (e.g. dendritic cells) are highly phagocytic but do not present antigen particularly well. During the innate immune response and after phagocytosis of an antigen (e.g. a microorganism), dendritic cells mature and present antigen very well to T lymphocytes. (Microorganisms and their products are particularly good at inducing the maturation of dendritic cells.) In addition, mature dendritic cells express on their cell surfaces large amounts of co-stimulatory molecules, e.g. B7 (also known as CD80 and CD86), and can also produce large quantities of cytokines required for T lymphocyte proliferation and differentiation. Immature dendritic cells do not express large amounts of co-stimulatory molecules or cytokines.

- Activation of a naïve T cell by antigen requires two signals. The first signal is the presentation of peptides by MHC and the second signal is the interaction between B7 on the APC and CD28 on the membrane of the T cell. These two signals lead to T cell activation (See **Figure 13.**) The regulated expression of co-stimulatory molecules ensures that naive T lymphocytes are activated only at the correct time and place.

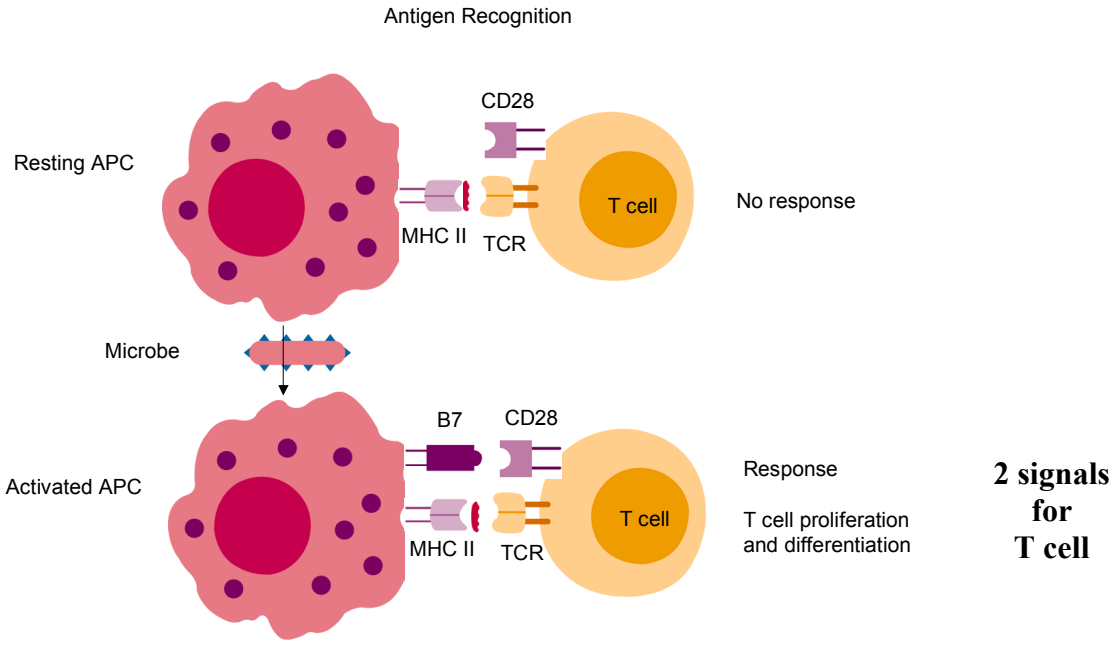


Figure 13. T cell activation requires a co-stimulatory signal provided by antigen presenting cells (APCs).

ACTIVATION OF T CELLS BY INTERACTION WITH ANTIGEN PRESENTING CELLS THAT DO NOT EXPRESS B7

- APCs that have taken up an antigen (which is not a microorganism) do not necessarily express B7. However, T cells that recognize peptides expressed by MHC class II on the surface of the APC are stimulated to express CD40 ligand, CD40L (also known as CD154). CD40L engages CD40 on the surface of the APC and this signal induces the expression of B7 by the APC.

- CD28 ligation of B7 induces T cell proliferation and differentiation. See **Figure 14.**

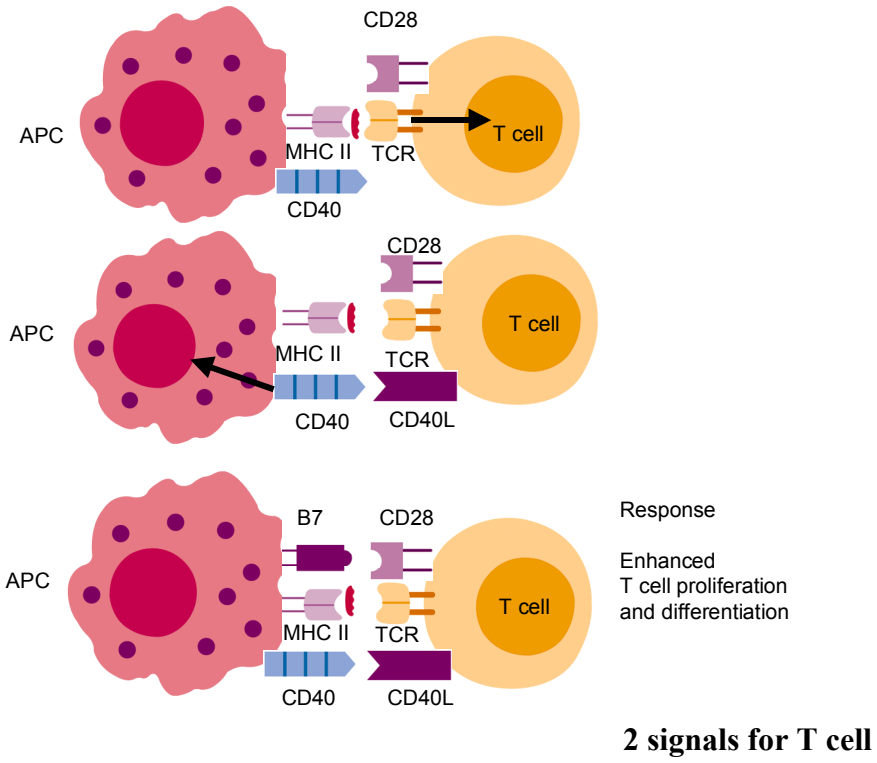


Figure 14. T cell induction of B7 on the APC surface.

CELLULAR REACTIONS REQUIRED FOR PRODUCTION OF ANTIBODIES

- Production of antibody to most antigens requires not just B cells but also T cells.
- B cells take up antigen, then B cells process antigen and display processed peptides on MHC class II molecules. This process activates the B cell to make B7. T cells recognize MHC presented antigen and B7 co-stimulates CD28 on the surface of the T cell to activate the naive T cell. This activation induces the expression of CD40L. CD40L engages CD40 on the surface of the B cell, activating the T cell to produce cytokines, allowing the B cell to proliferate and differentiate into plasma cells that secrete antibody. See **Figure 15.**

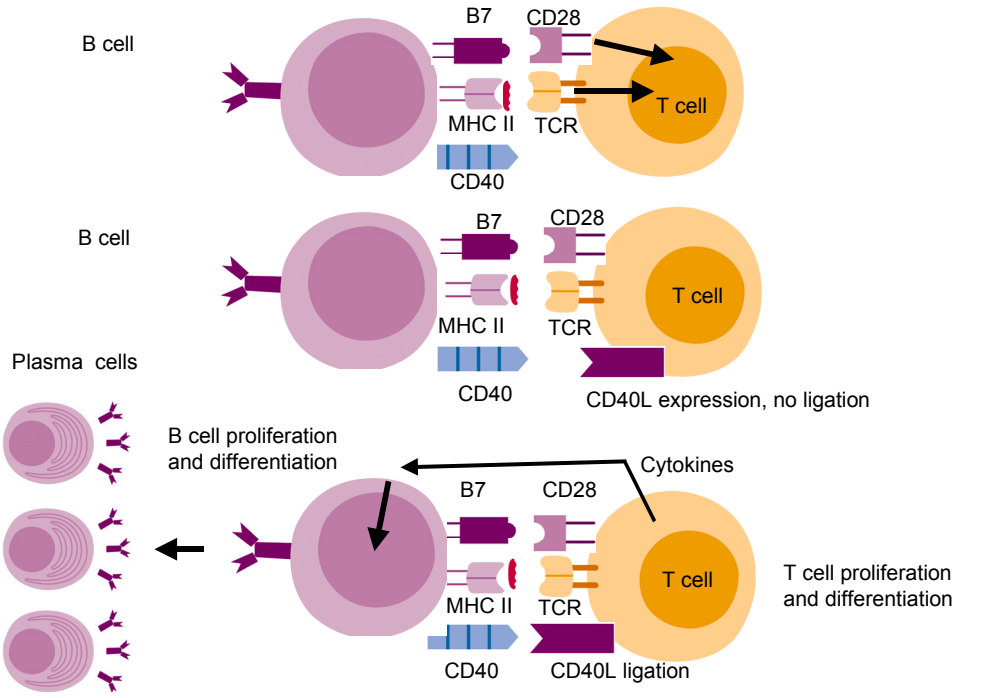


Figure 15. T cell mediated B cell activation.

IMMUNE SYNAPSE

- The activation of T lymphocytes is mediated by; the interaction of T cell antigen receptors (TCRs) with their ligands (major histocompatibility molecule-peptide complexes, MHC-peptide), and by a specific co-stimulatory signal like CD28 and B7 or CD40L and CD40.
- Within seconds of MHC-peptide engagement, the TCR initiates a phosphorylation cascade that triggers multiple branching signaling pathways.
- These early signals may be sufficient to trigger some effector functions, such as killer T cell execution of target cells.
- In contrast, more complex functions, such as T cell proliferation, require TCR engagement and signaling for many minutes or hours. The mechanisms of sustained TCR engagement is the formation of a specialized contact, termed the immunological synapse.
- The mature immunological synapse is defined by a specific pattern of receptor segregation with a central cluster of TCRs surrounded by a ring of adhesion molecules (like LFA-1).

- The formation of the immunological synapse provides a mechanism for sustained TCR engagement and signaling. An immunological synapse is shown in **Figure 16** and is comprised of a central cluster within concentric rings.

- The intermediate ring is enriched in adhesion molecules (like LFA-1-ICAM-1 complexes) that promote efficient TCR-MHC-peptide interaction leading to biological response. LFA-1 is on the T cell surface and I-CAM-1 is on the APC surface.

- The inner circle contains TCR, CD4, and co-stimulatory molecules (like CD28). The immunological synapse provides a higher-order molecular mechanism of junction formation, MHC-peptide transport, and cluster stabilization.

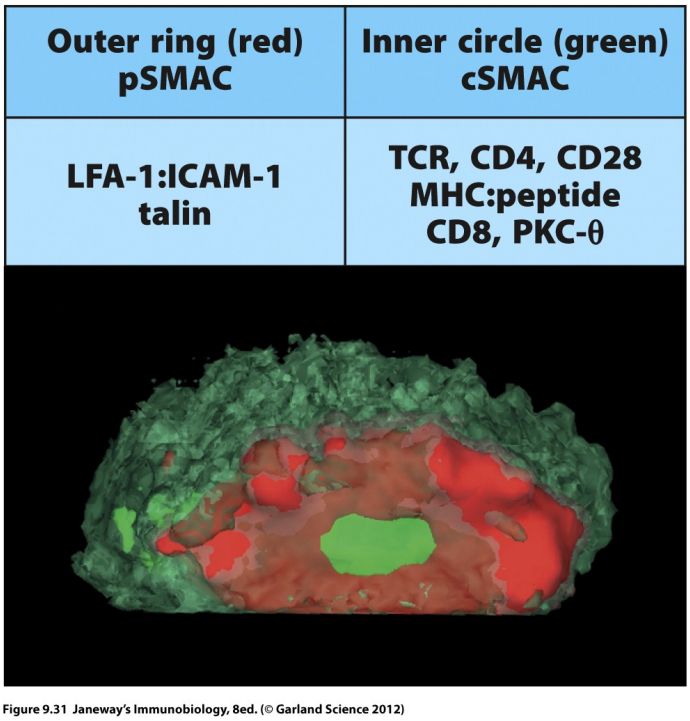
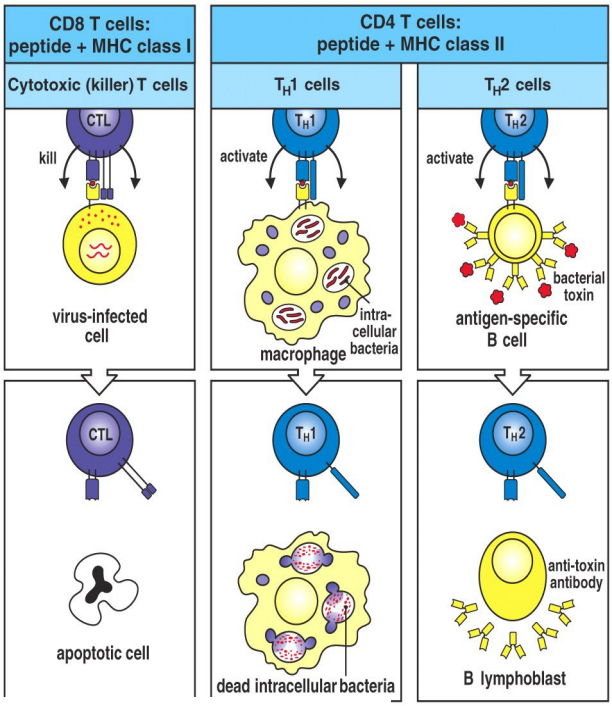


Figure 16. Immune synapse formation. (Text Movie 9.6 is not assigned but is well done and illustrative of the process.)

An overview of the immune response is presented in **Figure 17**.



To a lesser extent T_H1 cells can also activate B lymphocytes to produce antibodies.

Figure 17. Overview of cellular interactions of the immune response.

STUDY QUESTIONS

1. In order for T-cell recognition and activation to occur, a T cell needs to encounter a foreign antigen in association with an MHC protein. It would seem feasible for a single type of MHC protein to suffice for this purpose; however, as transplant surgeons have found to their dismay many different MHC proteins exist in humans. Why are MHC proteins so polymorphic; that is, why are there so many different MHC alleles?

2. Compare MHC restriction for T helper and cytotoxic T lymphocytes.

3. Compare the various types of antigen presenting cells.

EXAMPLE OF TEST QUESTION

Co-stimulatory molecule expressed on the surface of CD4+ T lymphocytes:

A. CD4.

B. CD8.

C. CD28.

D. CD95.

E. Non-polymorphic regions of class I MHC molecules.

CORRECT ANSWER TO ABOVE QUESTION: C

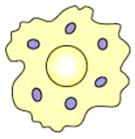
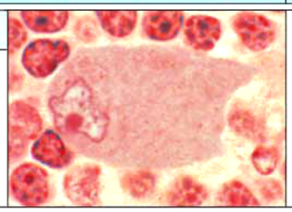
Antigen Presentation and the MHC

Herb Mathews

hmathew@lumc.edu

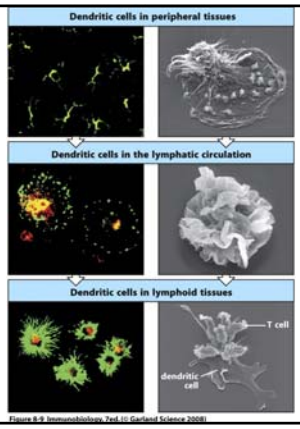
Table I. **Antigen Presenting Cells (APCs).**

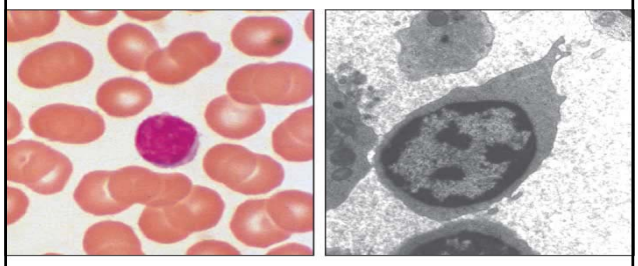
Cell Type	Location	Phagocytic	Class II
Mononuclear phagocytes (Macrophages)	Blood, liver, spleen tissues	Yes	Yes
Dendritic cells	Skin, lymphoid tissues	Yes	Yes
B lymphocytes	Lymphoid tissues, sites of immune reactions	No	Yes

Cell	Activated function
Macrophage  	Phagocytosis and activation of bactericidal mechanisms Antigen presentation

Liver-Kupffer cells
Brain- Microglial cells

Skin- Langerhans' cells
T lymphocyte area of lymph nodes – interdigitating dendritic cells
B lymphocyte area of lymph nodes - follicular dendritic cells



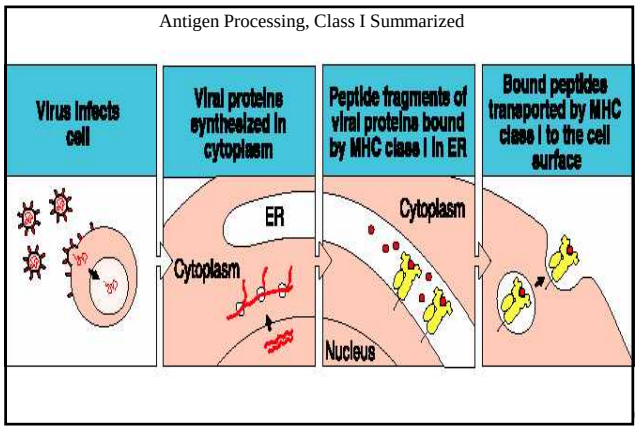


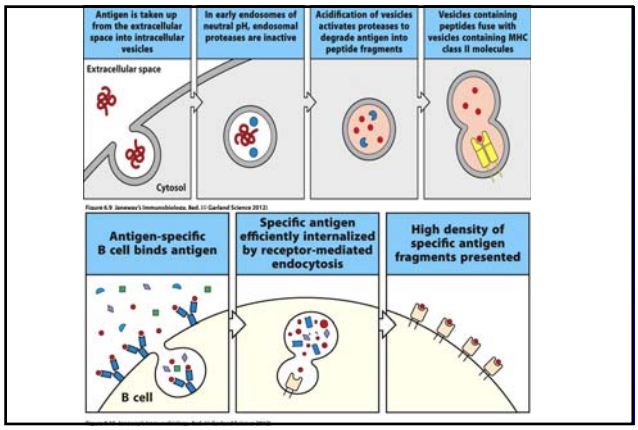
B lymphocytes

Antigen Processing

Summarized

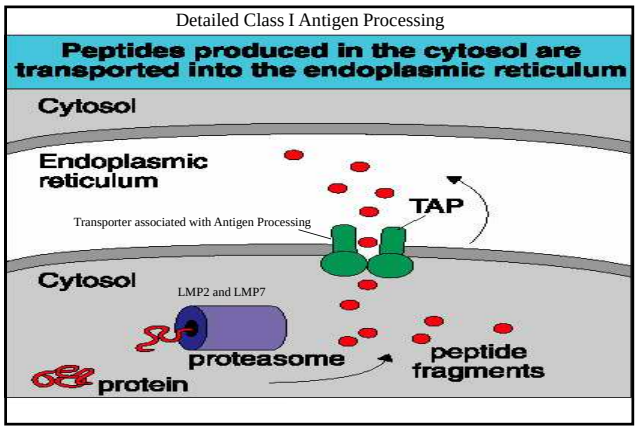
Antigen Processing, Class I Summarized

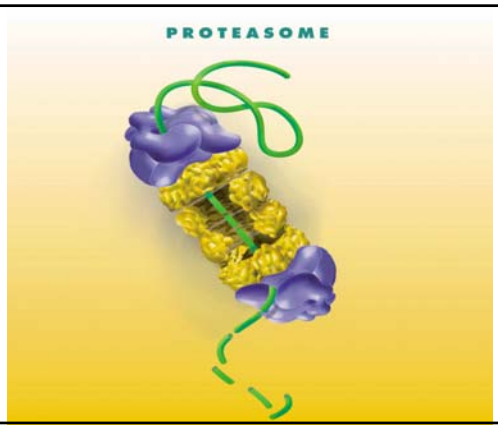


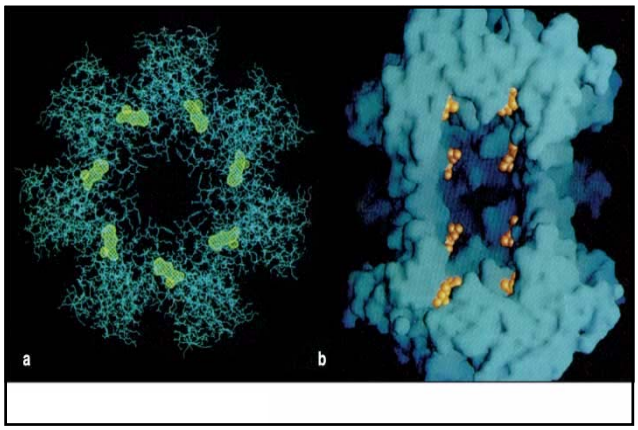


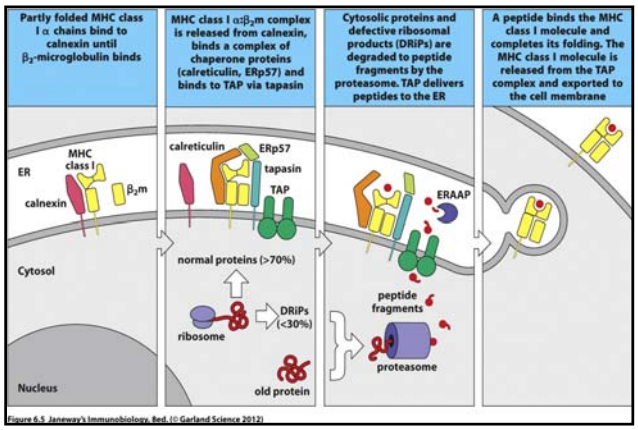
MHC Class I Antigen Processing

Detailed Class I Antigen Processing









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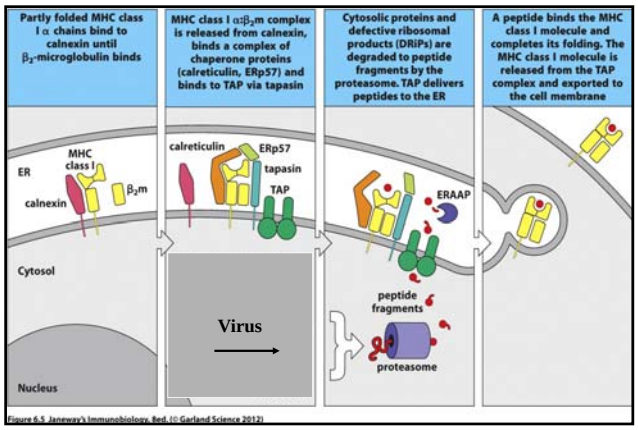

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MHC Class II Antigen Processing

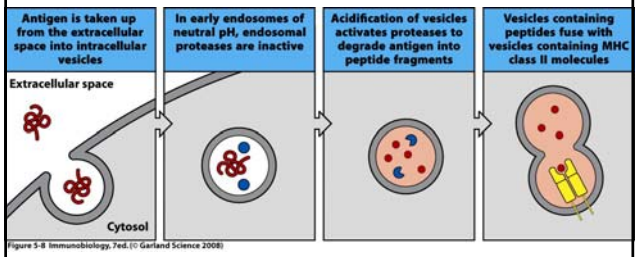


Figure 5-8 Immunobiology, 7ed. (© Garland Science 2008)

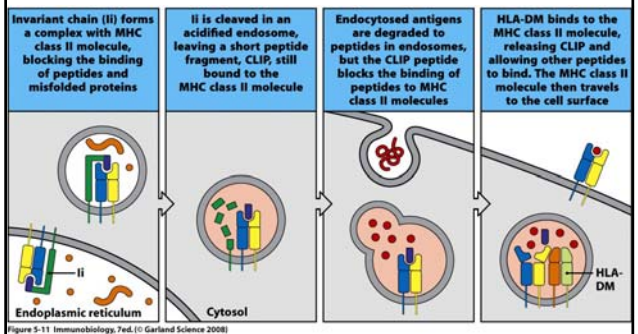
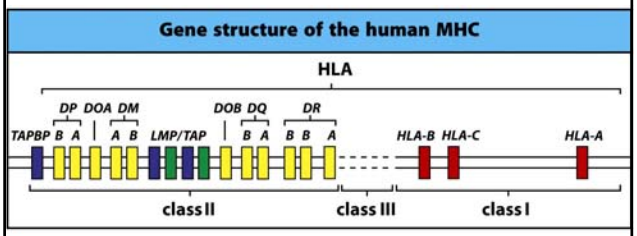
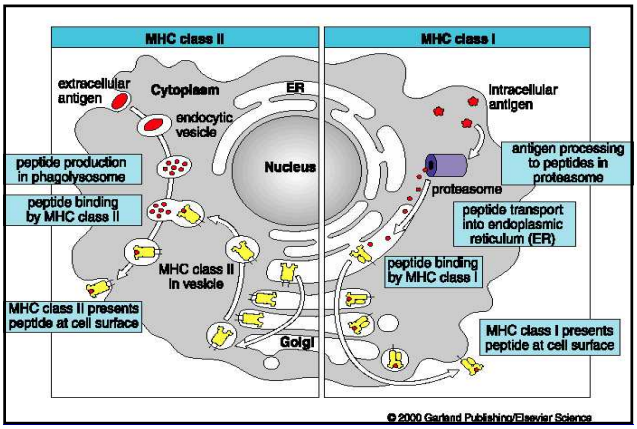


Figure 5-11 Immunobiology, 7ed. (© Garland Science 2008)

CLIP = class II associated invariant peptide

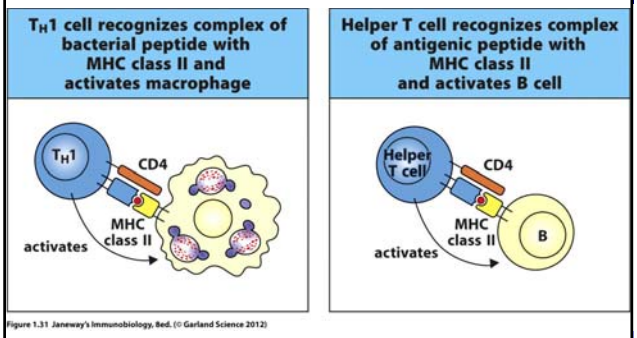
Figure 5.12





MHC Class II Restricted Presentation of Antigen to T Lymphocytes

- CD4+T lymphocytes do not recognize free or soluble antigens.
- Rather, they recognize antigen on the surface of APCs in the context of class II molecules.
- The interaction between T lymphocytes and antigen and the class II molecule is highly specific and will result in T cell proliferation and differentiation.
- The CD4 subset of T lymphocytes contains effectors for:
    - Macrophage activation - designated Th1.
    - Antibody synthesis - designated Th2.



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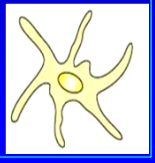
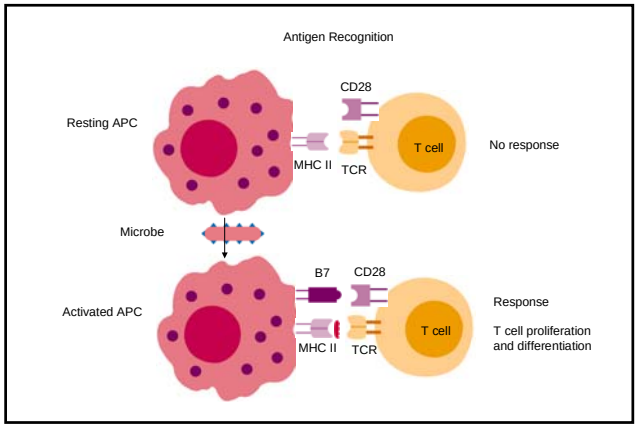
T Lymphocyte Activation

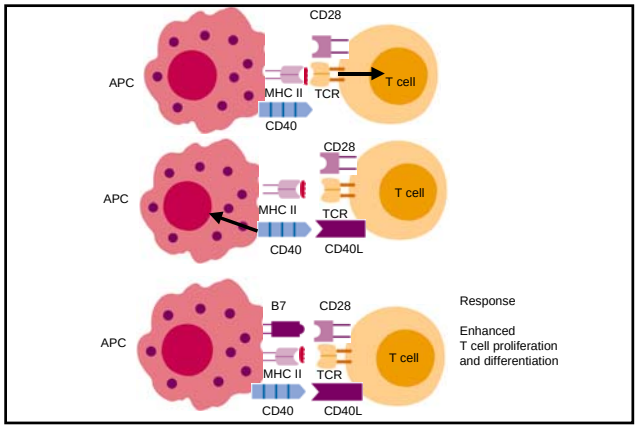
Activation of Naïve T Cells by Interaction with Antigen Presenting Cells

page 10

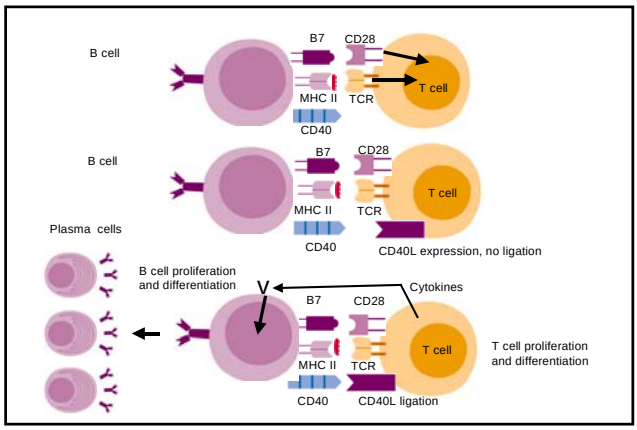




Activation of T Cells by Interaction with Antigen Presenting Cells That Do Not Express B7



Cellular Reactions Required for Production of Antibodies



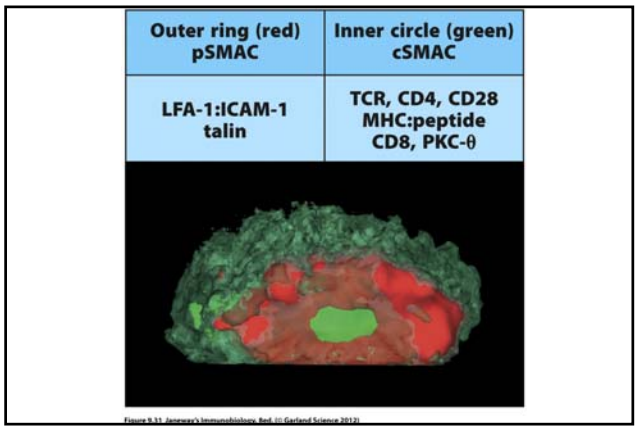
Immune Synapse

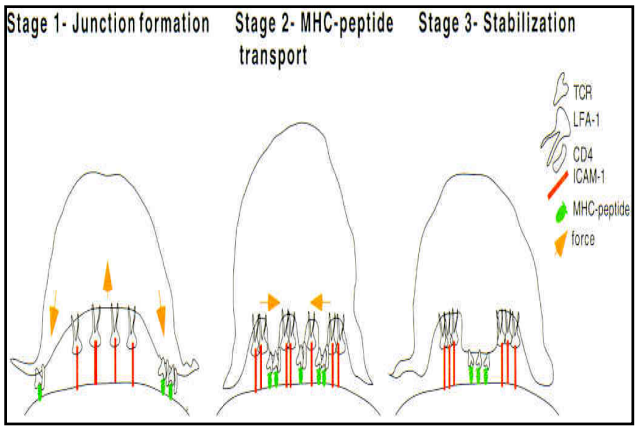
page 13-14

Complex functions, such as T cell proliferation, require TCR engagement and signaling for many minutes or hours.

The mechanisms of sustained TCR engagement is the formation of a specialized contact, termed the immunological synapse.

The formation of the immunological synapse provides a mechanism for sustained TCR engagement and signaling.





Outer ring (red) pSMAC	Inner circle (green) cSMAC
LFA-1:ICAM-1 talin	TCR, CD4, CD28 MHC:peptide CD8, PKC-θ

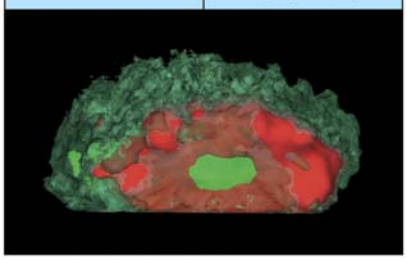


Figure 9.11 Janeway's Immunobiology, 8ed, (© Garland Science 2012)

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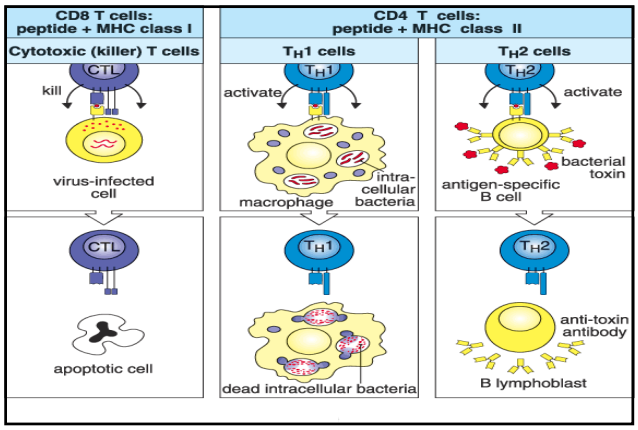


STUDY QUESTIONS

In order for T-cell recognition and activation to occur, a T cell needs to encounter a foreign antigen in association with an MHC protein. It would seem feasible for a single type of MHC protein to suffice for this purpose; however, as transplant surgeons have found to their dismay many different MHC proteins exist in humans. Why are MHC proteins so polymorphic; that is, why are there so many different MHC alleles?

Compare MHC restriction for T helper and cytotoxic T lymphocytes.

Compare the various types of antigen presenting cells.

HOST DEFENSE

SMALL GROUP PROBLEM SOLVING SESSION

CLINICAL IMMUNOLOGIC ASSAYS-I

Small Group Classrooms

LEARNING GOAL

Understanding in vitro assessment of immunologic responses that have contemporary clinical relevance

BACKGROUND READING

Janeway 8th: Most of the concepts are developed in the text of the Notes, Dr Knight's lectures, and also the Letter to the Editor and a Clinical Implications of Basic Research from the *New England J of Medicine* that are posted on the HD site.

DEVELOPED BY

John A. Robinson, M.D.

Rev 11/01/2012

I. Active and Passive Immunity

A. Introduction

1. Many life-threatening infections are caused by the release of protein toxins by bacteria after they have infected a patient. *Diphtheria* and *tetanus* are two prime examples. A patient will be protected against the toxin effects if specific antibodies against the toxin are present at the time of infection.
2. Protective antibodies arise during actual infection or can be induced by vaccines
3. The following vignettes underscore the need to understand basic immune responses and their timing in order to successfully treat some serious infections.
4. Implementation of an immunologic treatment strategy must be based on understanding of how specific antibody responses are induced and how long they are effective.
5. For the purposes of this Small Group, do not worry about the complex cellular interactions required for antibody formation - this comes later but be sure you understand the CONCEPTS of primary and secondary immunization and active versus passive immunization

B. The following graph illustrates the temporal sequence of antibody formation to an antigen (or infection).

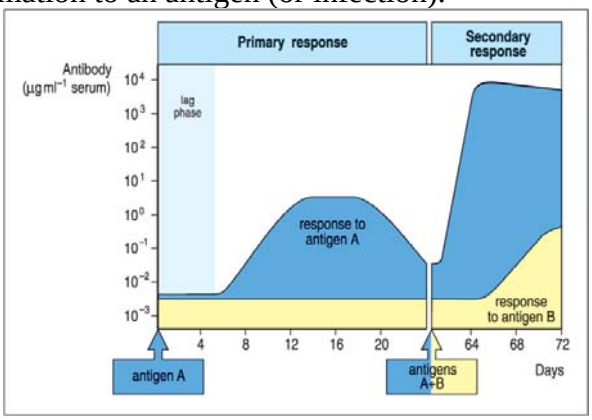


Fig 1.20 © 2001 Garland Science

Fig. 1.20 The course of a typical antibody response. First encounter with an antigen produced a primary response. Antigen A introduced at time zero encounters little specific antibody in the serum. After a lag phase, antibody against antigen A (blue) appears; its concentration rises to a plateau, and then declines. When the serum is tested for antibody against another antigen, B (yellow), there is none present demonstrating the specificity of the antibody response. When the animal is later challenged with a mixture of antigens A and B, a very rapid and intense secondary response to A occurs. This illustrates immunological memory, the ability of the immune system to make a second response to the same antigen more efficiently and effectively, providing the host with a specific defense against infection. This is the main reason for giving booster injections after an initial vaccination. Note that the response to B resembles the initial or primary response to A, as this is the first encounter of the animal with antigen B.

C. The temporal lag in appearance of specific antibody in the patient's peripheral blood **during a primary immune response** to an infectious pathogen is a critical problem. The early period of little to no antibody production is a time of extreme vulnerability. Antibiotics provide some protection while the immune system gears up for antibody synthesis which then provides **active immunity.**

D. The significant decline of serum antibody concentration over a 4-6 week period after an immune stimulus emphasizes the importance of memory in adaptive immune responses. The memory pool of immune cells that remember the first infection prime the system for a rapid, specific response to the same pathogen. This is designated a **secondary immune response**.

E. The graph also illustrates the rapidity of a secondary response to the same antigen that the individual had originally been vaccinated with. Not only is there is **no** "window" of opportunity for organism and its toxin to escape antibody neutralization after the second antigen exposure but serum antibody levels are maintained thereafter for a much longer period of time.

F. Vaccines are designed to **mimic active immunity** that would have been triggered by an actual infection. Use of a vaccine before the actual infection provides the host with the ability to respond with a rapid secondary response that abbreviates the vulnerable period of no antibody formation during early infection

G. Modern biotechnology now provides a way for the physician to provide "instant" specific antibodies to an infected or potentially infected patient. In this case, the physician, using epidemiologic and clinical data, must be able to predict the organism that the patient is most likely infected with and then **passively immunize** the patient by infusing the specific, premade antibody into the patient at the time of the infection. The drawback to passive immunization is that the duration of protection against the infection is defined by the half-life of the infused antibody which is about 3 weeks. **With a few exceptions, no active immunization occurs** (you may see an example of the exception later in this exercise).

H. Apply the **basic concepts** of primary and secondary immune responses and active and passive immunity to the clinical examples below (You do not have to read about the specific diseases to understand the concepts). What type of immunity, if any, is needed

for in order for the following 2 patients to resist infection?

1. A twenty year old on active military duty arrives at your office with a one inch long, very superficial laceration of the forearm caused by a dog bite. The dog belonged to his neighbor and was able to be confined and observed for clinical rabies. The real concern is whether the patient could die from tetanus toxin if a tetanus infection transpired. Tetanus bacteria only grow well in situations where there is reduced oxygen- e.g., a deep penetrating wound, like a nail in a foot. This wound was easily cleaned and did not require sutures.

2. A 23 year old mountaineer, who arrived in town only last week, shows up in the ER with a lacerated, swollen and tender swelling of the right gluteus muscle. The wound has dirt and leafy debris embedded in it. The patient was bitten while "escaping from an angry bear". He lived in a “holler” his entire life and has never been to a doctor before. Knowing what you do about tetanus from the first clinical scenario, what immunologic concepts would have to be applied in this instance?

I. Now apply your general understanding of immune responses to the following clinical problems.

1. If the **anthrax organism** gains access to a host environment that allows it to move from the spore form to a toxin producing phase, the patient may die very rapidly because of the severe systemic effects of its toxins. Anthrax produces three exotoxins. Two of them, after gaining access to the interior of host macrophages and monocytes, kill them. The dead cells release massive amounts of molecules that have potent and ultimately deadly effects on coagulation and cardiovascular responses. The other toxin,

called a shuttle vehicle, delivers the 2 lethal toxins to the host cells.

a. Predict and discuss what type of immunity must be generated and the most rational strategies to provide immunologic protection against anthrax.

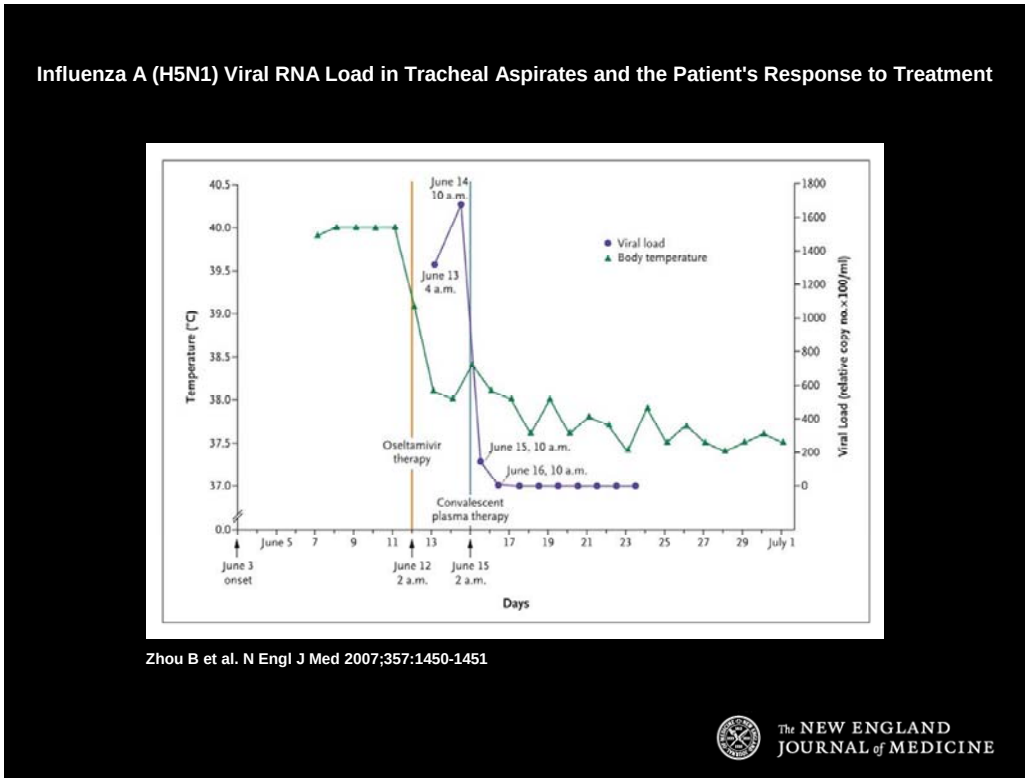
2. Recently, hundreds of campers from all over the USA who had camped in a Yellowstone park campsite were potentially exposed to a lethal virus (Hanta). Months later, epidemiologic analysis of the clinical cases of pneumonia caused by the virus by the National Communicable Disease Center revealed that the route of infection was respiratory via rodent feces found in several camphouses. All campers that were known to have stayed in those houses were contacted and serum samples were collected from them. What findings in those samples would be reassuring and why?

3. A large group of African patients are known to have survived Ebola virus infection. This is a rapidly progressive infection with a very high fatality rate and currently there is no vaccine available for it. Your research lab in Kenya is studying antibody responses to Ebola and has large amounts of plasma from Ebola survivors.

a. If Ebola virus infection broke out in your lab, what type of immunity would have to be invoked and how could you do it?

J. The slide below depicts a real-life application of the concepts you have just discussed. The entire “letter to the editor” is posted on the Host Defense Website. The clinical

problem was that a patient in southern China developed a life threatening case of avian influenza. The virus had caused similar cases in the region but they were not all fatal. Plasma (which will contain antibodies only, no leucocytes) from a survivor was infused into the patient. Interestingly, the antibody levels to the avian influenza virus remained high for 6 months after the patient recovered. What type of immunity was operative in this success story?

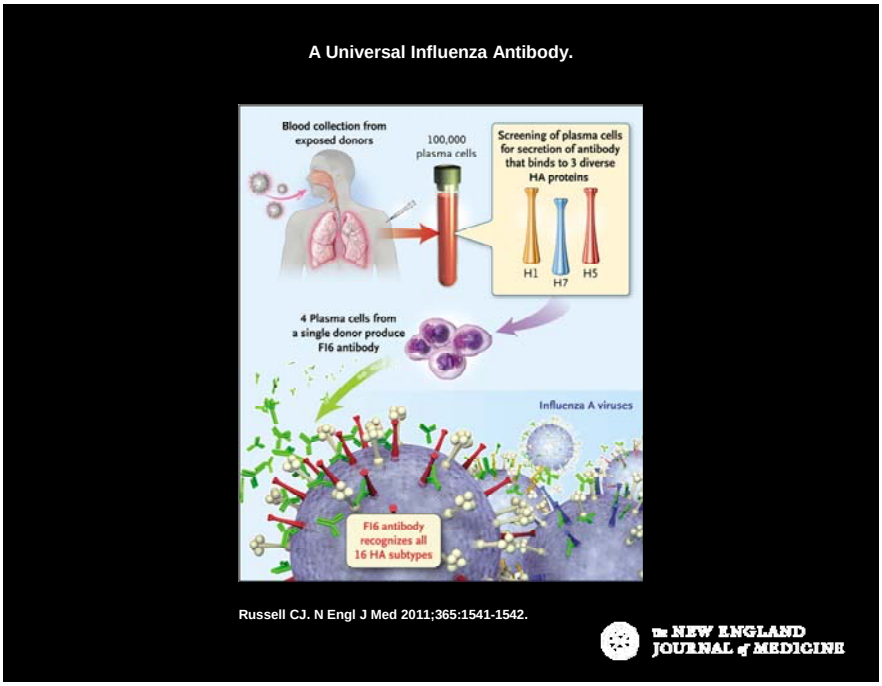


L. In the previous example in J, the patient did well because the plasma he was given contained antibodies specific for the same virus that had infected the survivor. If that plasma had been given to a patient with influenza the **following** year it might not have been effective because unfortunately the influenza virus is able to constantly change the structure of its surface proteins

and escape the immune system. This is why you get a new version of influenza vaccine every year.

1. There is a desperate need for a universal influenza vaccine that could induce long lasting antibodies effective against all flu strains, even pandemic ones .If this can be achieved, the need for annual development of a new vaccine and annual vaccination would be obviated.

2. How close is this and how did the use of crystallography provide a way to potentially eliminate yearly flu vaccines? You will have to read the "Clinical Implications of Basic Research" posted on the HD website (fig. below) to discuss it intelligently



II. Immunologic Assays used for detection and assessment of antibody responses that students, residents and attending physicians have to interpret frequently

A. General Principles

1. What does the term "antibody titer" mean?
   a. Titer is an old but still frequently used term that serves a purpose because it provides a sense of the amount of antibody to the antigen measured in the patient's serum or whatever biological fluid measured; for example, spinal fluid, urine, pleural fluid. Many

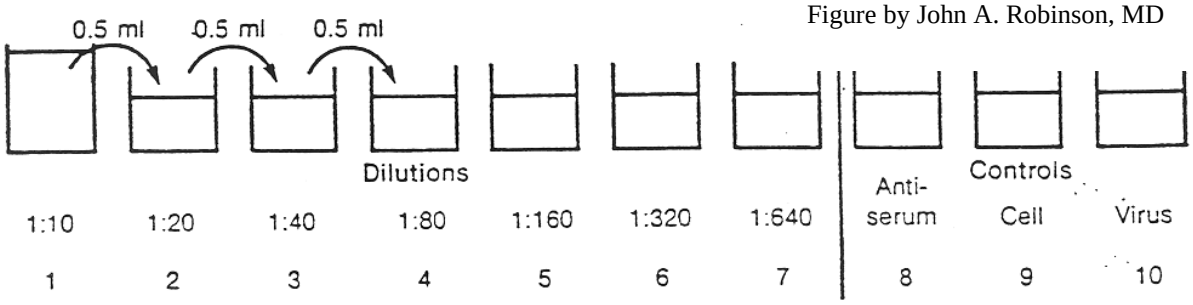
new clinical antibody assays now express the titer concept in "units".

b. The principle is: the greater the amount of antibody in the serum, the further the serum can be diluted and antibody still be detected.

c. How is a "titer" determined? The following is an example of the basic procedure.

Prepare serial twofold dilutions of the serum to be tested for antibody to the antigen ranging from 1:10 to 1:640 in 0.5ml amounts as shown below.

Figure by John A. Robinson, MD



Begin with 1.0 ml of a 1:10 dilution of serum in well 1 and 0.5 ml of saline in wells 2 through 7. Remove 0.5 ml from well 1, place it in well 2, and mix. You now have 1.0 ml of a 1:20 dilution in well 2. Next, take 0.5 ml of the 1:20 dilution and mix it with the 0.5 ml of saline in well 3. This gives 1.0 ml of a 1:40 dilution of serum in well 3 and leaves 0.5 ml of a 1:20 dilution in well 2. Repeat this procedure for wells 4, 5, 6, and 7 to give serial twofold dilutions in the row of wells.

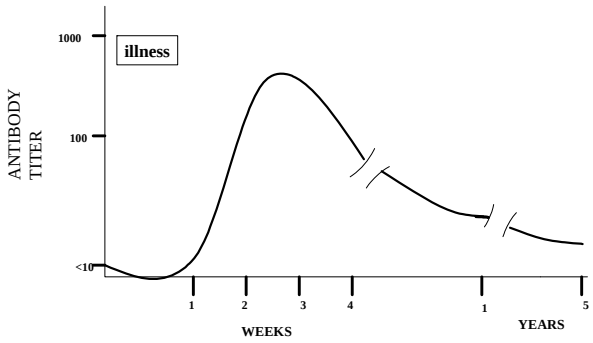
d. Study the response to an influenza virus infection shown below. The usefulness of the titer concept should be evident. At the time of infection, the patient had no specific antibody to the virus and became ill. The response of the immune system can be tracked by the rising titers to the virus. By 2 weeks the titer was ~1/100 and at 3 weeks it peaked at 1/500. A year later, the patient still had residual amounts of specific antibody but at a much lower titer.

e. Titers can be very helpful in several ways:

1. If, **at the beginning of an infection**, a patient already has **IgG** titers to the suspected virus, you know that the suspected virus is not the cause of the current illness but that the patient, **sometime in the past**, has been infected with it.

2. If, **at the beginning or during the clinical expression of an infection**, no

SEROLOGICAL RESPONSE (IgG) DURING A VIRAL INFECTION



anti-viral IgG can be detected but a **titer of IgM** specific for the virus is detected, it is very likely the patient has been infected very recently with the virus in question.

1. Questions

a. Three patients (A, B, C) have the following symptoms: chills, fever, muscle aches and cough (all typical of a "flu-like" syndrome)
   i. "**A**" symptoms are markedly better but he came to clinic anyway about 18 days after he got sick.
   ii. **"B"** has had the symptoms for about 48'
   Iii. "**C**" has been sick for 2 weeks

The physician suspects they all have (or had) Virus X and draws blood from them at the time of their visit (Day 1), 3 weeks later (Day 21) and then a year later (Day 365) and has them tested for IgM and IgG antibodies to Virus X. Appropriate positive (known serum positive for antibodies to X) and negative (known serum negative for X antibodies) **controls** were done.

Results:	*D1*	*D21*	*D365*
"A" IgM	**1:10**	**1:2**	**Negative**
IgG	**1:100**	**1:300**	**1:20**
"B" IgM	**1:20**	**1:50**	**Negative**
IgG	**Negative**	**1:300**	**1:10**
"C" IgM	**Negative**	**Negative**	**Negative**
IgG	**1:50**	**1:50**	**1:50**

Did everyone have virus X and, if they did, when?

B. **The ELISA test** has become a common way to screen sera for specific antibodies. As a general rule, it **is highly sensitive** to the detection of antibodies, but may have **low specificity** for a specific pathogen. Clinically, a **negative** ELISA is very helpful because it effectively excludes the presence of the infection being considered (assuming the patient is able to make antibodies). A positive ELISA however almost always prompts more specific immunologic testing to validate the ELISA result.

1. **ELISA (enzyme linked immunosorbent assay)** exploits the ability of certain enzymes to form color compounds after reacting with specific substrates. These enzymes can be linked to antibody probes. The enzyme tagged antibodies are then incubated in systems where the antigen in question has already been bound to a surface (usually a plastic well). If a specific reaction occurs, the **colorless substrate specific for the enzyme** that was used as a tag on the probe antibody is then added and a color reaction will develop if the tagged antibody had bound to its antigen and the color reaction can be quantitated.

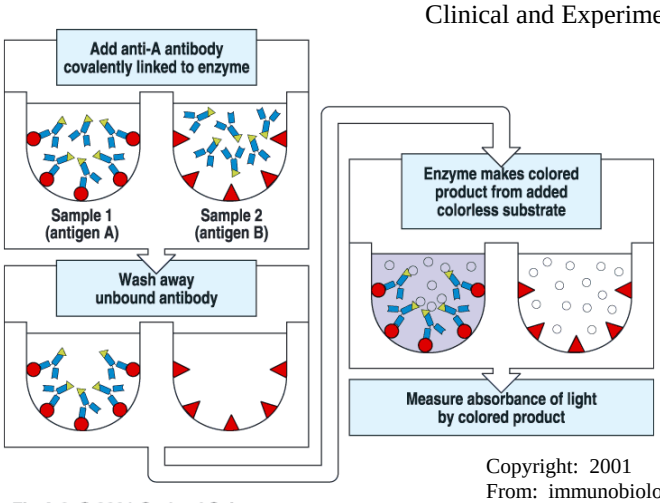


Fig A.6 © 2001 Garland Science

2. The **ELISA method** is reaction of a specific antigen (viral, bacterial, etc.) bound to a plastic surface with an unknown serum to determine if the serum contains specific antibodies to the antigen in question. After excess serum is washed away, enzyme linked **antihuman Ig** is added to detect any bound patient Ig to the antigen. After washing again, a **substrate** specific for the enzyme that had been tagged onto the antihuman Ig is added. If specific antihuman Ig plus patient Ig plus antigen X is present, color develops. Several variations of this test can be used clinically but the principle remains the same.

3. An example of the ELISA "problem": A medical student draws blood from a combative and agitated patient in the emergency department. During the procedure the patient knocked the syringe out of the student's hand and the needle tip punctured her forearm. The patient's blood was sent to Pathology for HIV testing. An ELISA assay was done (see below) and was "indeterminant."

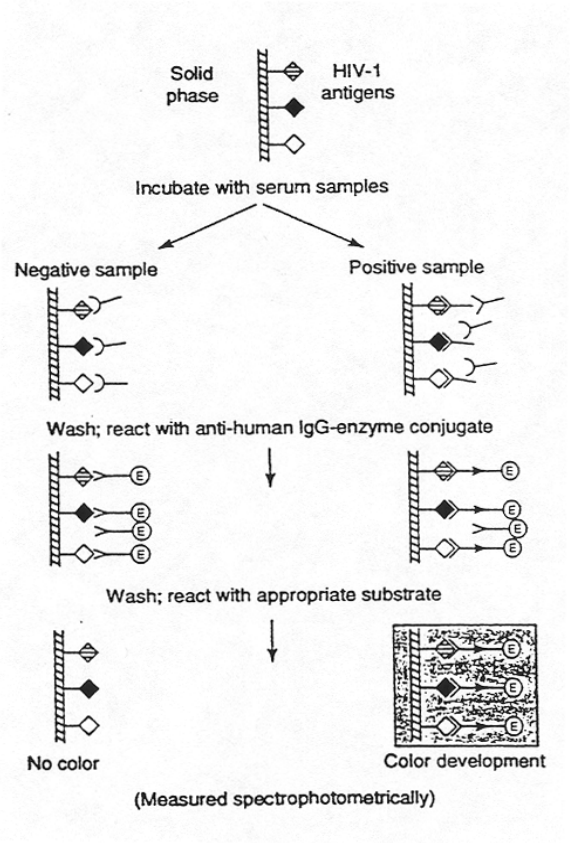


Figure by John Robinson, MD

4. A Western Blot of the patient's serum was then done. Be sure you understand why a Western Blot is necessary. To do this you must understand the concepts **of sensitivity and specificity** of laboratory assays

5. IMMUNOBLOT - WESTERN BLOT ANALYSIS

a. This test exploits the ability of an electrical charge to 'pull' a mixture of antigens through a porous gel. Smaller antigens migrate rapidly through the gel; larger ones migrate slowly or not at all. After a fixed time under the influence of the electrical field, the migrated antigen bands are then transferred by direct apposition to a membrane (usually electrostatically) and can then be localized and identified with specific antiserum layered near the membrane. The antigen-antibody reactions are then visualized by using a developing agent that usually is antihuman Ig and a chromogenic substance. Substituting an unknown (patient serum) as the source of viral specific Ig and then comparing the specific bands to known antigen controls is a sensitive test for specific antibody.

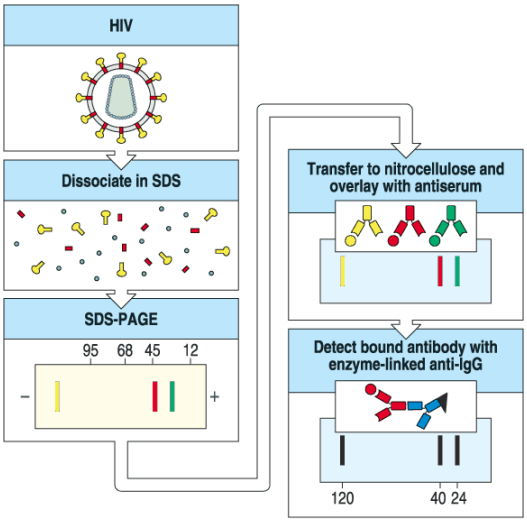


Fig A.20 © 2001 Garland Science

b. Western blot analysis is used to **confirm** the presence of anti-HIV antibody in a patient who has a positive ELISA assay for HIV antigens. The sequence here would be: HIV **antigen mixture** plus electrophoresis results in migrated HIV proteins. Add patient sera to be tested and a positive anti-HIV control, incubate with antihuman Ig and a colorimetric compound, which then is developed for confirmation of a specific antibody response to HIV.

DEVELOPMENT OF T LYMPHOCYTES

Reading assignment: Janeway (8th Edition): 247-251; 290-315.

LEARNING GOALS:

Understand the relationship between T lineage commitment, differentiation, selection process and maturation of different subsets of T cells in the human thymus.

OBJECTIVES: You will be able to:

1. Interrelate thymic microenvironment with multiple steps in T cell development.
2. Identify and correlate cell surface markers with T subsets and their development.
3. Understand the differences between β selection, negative and positive selection processes in T cell development.
4. Understanding the mechanisms in T cell development that establish self and non-self recognition in T cells.
5. Recognize and understand different subsets of T cells developed in the thymus and their respective function.

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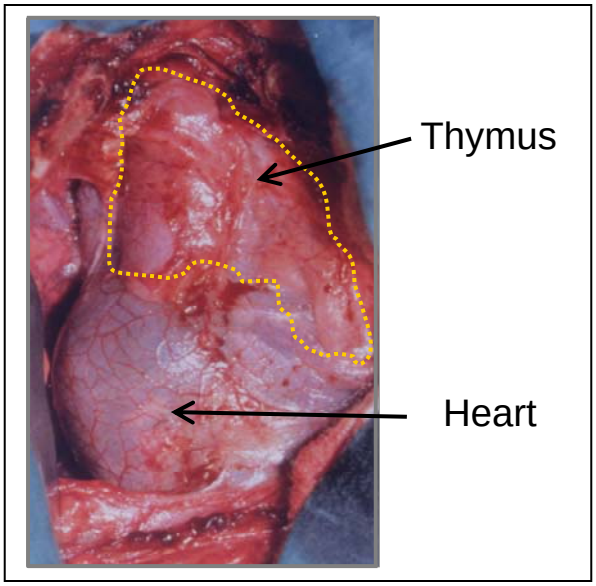
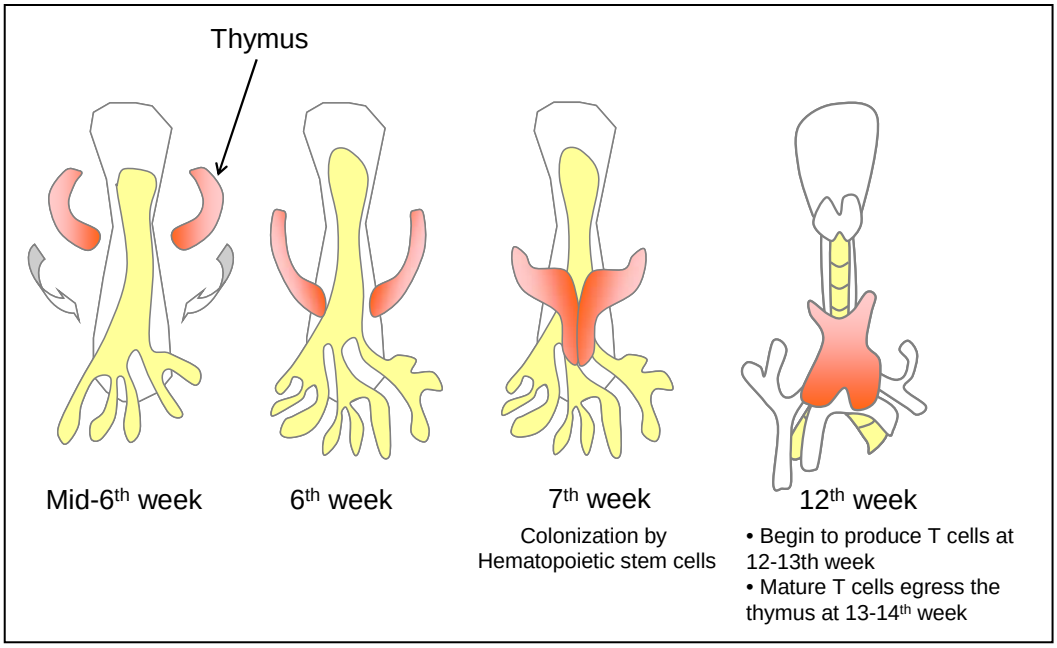
I. Thymic Development in Utero

The thymus is an epithelial-lymphoid organ that develops very early during embryonic development. Hematopoietic stem cells (HSC) home to the thymus and develop into: T helper (CD4), cytotoxic (CD8), natural killer T (NKT) and T regulatory (T_{reg}) cells.



The epithelial component of the thymus derives bilaterally from epithelium of the third pharyngeal pouch at fourth week of gestation. Between 4-7th week of gestation, the primordial thymic glands lose connections with the pharynx and migrate to the final position in the mediastinum forming a single bilobate gland.



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- The normal human thymus develops early on in fetal development; the glands are colonized by hematopoietic stem cells at 7-8 weeks of gestational age.
- The thymus begins to produce T cells around 12-13 week of gestation.
- Mature T cells egress the thymus and colonize peripheral lymphoid organs at the end the 13 and the beginning of the 14 week of gestation.
- ***Thus, by the time the baby is born, the peripheral T cell repertoire is established to the point that thymectomy does not cause immediate immune deficiency.***

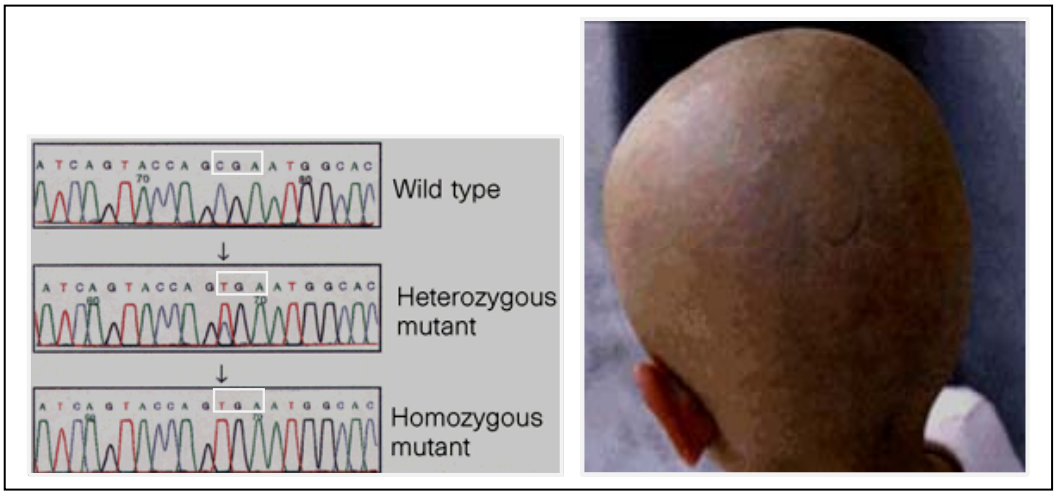
II. Genetic Evidence for Thymus as the Organ in which T Cells Develop

1. DiGeorge syndrome (DGS): Velo-Cardio-Facial Syndrome (VCSF).

DiGeorge syndrome is caused by a large deletion in chromosome 22 which is caused by an error in recombination at meiosis.

- It is a rare congenital (i.e. present at birth) disease syndrome whose symptoms vary greatly between individuals but commonly include a history of heart defects, characteristic facial features and recurrent infection due to absence of the thymus and T cells.
- The absence of the functional thymus results in complete T cell deficiency and severe immunodeficiency.
- Transplantation of an allogeneic thymus graft into patients with DGS rescues T cell deficiency.

2. Mutation in the *FOXN1* gene: The *FOXN1* gene (on chromosome 17) encodes a transcription factor that is essential for the functional maturation of thymic epithelial cell progenitors.



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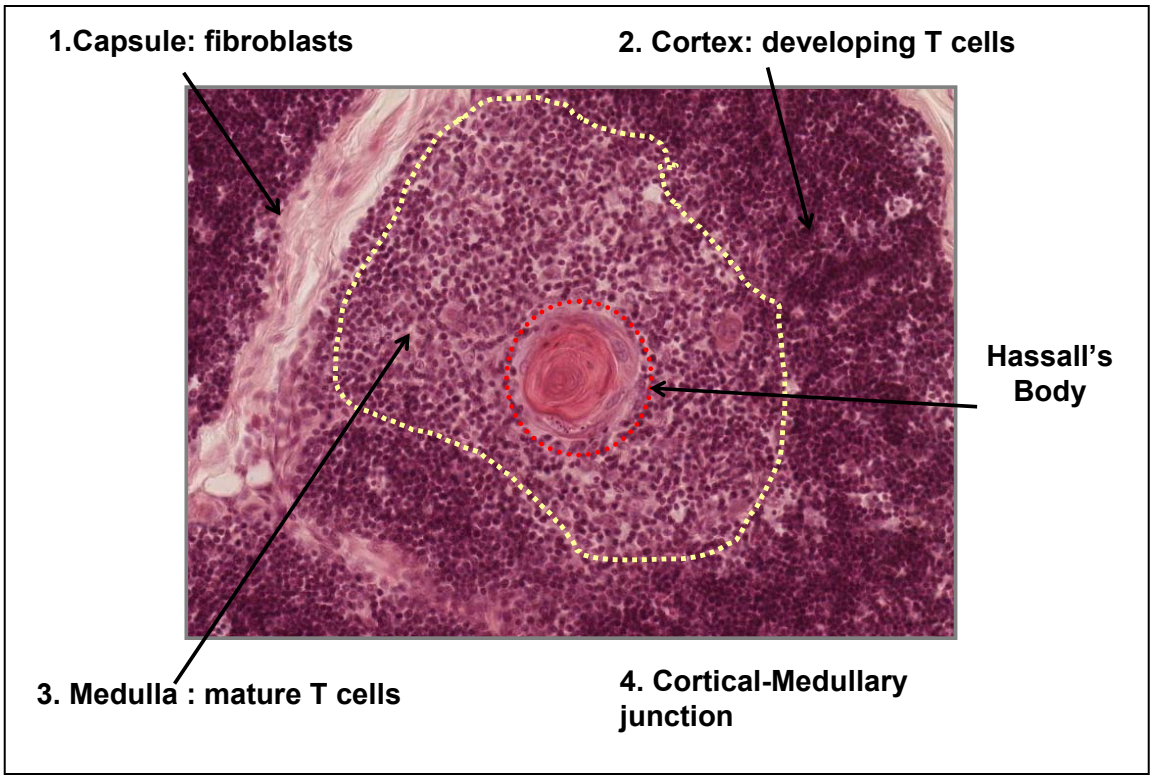
- In these patients, the thymus gland fails to form in utero because epithelial progenitor cells fail to undergo functional differentiation and instead form cyst-like structures with immature morphology.
- The immature epithelial cells fail to recruit hematopoietic stem cells into the organ.
- ***Thus, the functional maturation of thymic epithelial cells is required for the development of a normal thymic architecture, which is essential for the production of various thymic-dependent T cells subsets and the initial establishment of the peripheral T cell pool in animals and humans.***
- ***Thymic implant recently been shown to restore T cell immune response in patients with Foxn1 mutations***

III. Cellular Composition of the Thymus Glands



1. Thymic stroma: this includes the predominant thymic epithelial cells (TEC) and fibroblasts.

   a. Fibroblasts: found in the thymic capsule and septa.

   b. Thymic epithelial cells (TEC): provide three critical functions for the development of T cells.

      - TEC are identified as cortical TEC, medullary TEC and Hassall's TEC based on their anatomical locations within the thymic gland. All TEC are derived from endoderm.
      - TEC produce cytokines such as IL1, IL6, IL7, and SCF (stem cell factor), TSLP (thymic stroma lymphopoietin) that are requires for growth and differentiation of various immature T cells.

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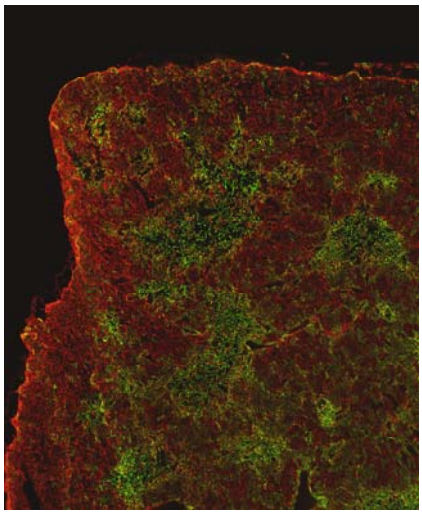
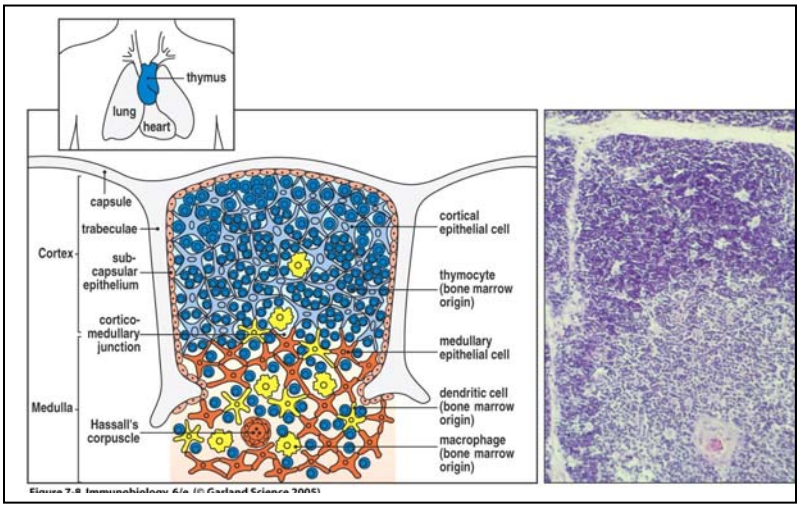
- TEC also express cell surface molecules such as ligands Delta-like-4 (DL-4) and DL-1 for the notch receptor. Signal of notch receptors expressed on progenitor cells is required for T cell lineage commitment.
- TEC expression of MHC classes I, and II/self antigen complexes controls the selection of maturing T cells.
- Expression of peripheral tissue antigens: for example insulin

Thymic epithelial cells of the cortex (red) and medulla (green) regions as seen with anti-keratin antibodies.



2. Macrophages and dendritic cells: these cells mature from the bone marrow and migrate into the thymus. These cells are scattered in the cortex and medulla; however, they highly populate the cortical-medullary junction. They function: in antigen presentation; deletion of autoreactive T cells (negative selection) and phagocytosis of apoptotic thymocytes.



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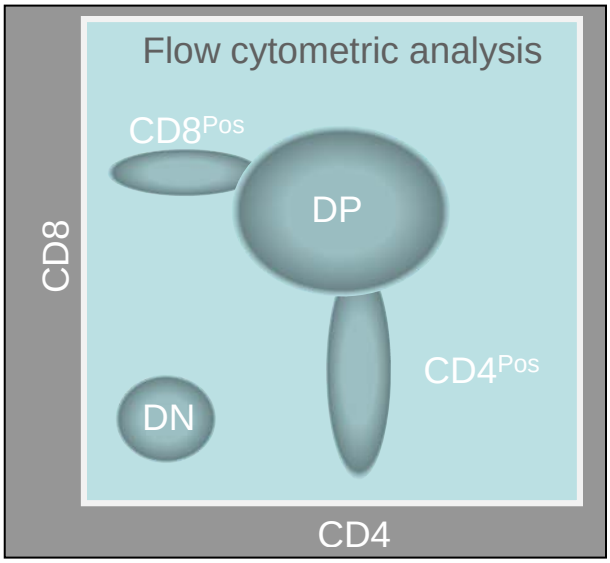
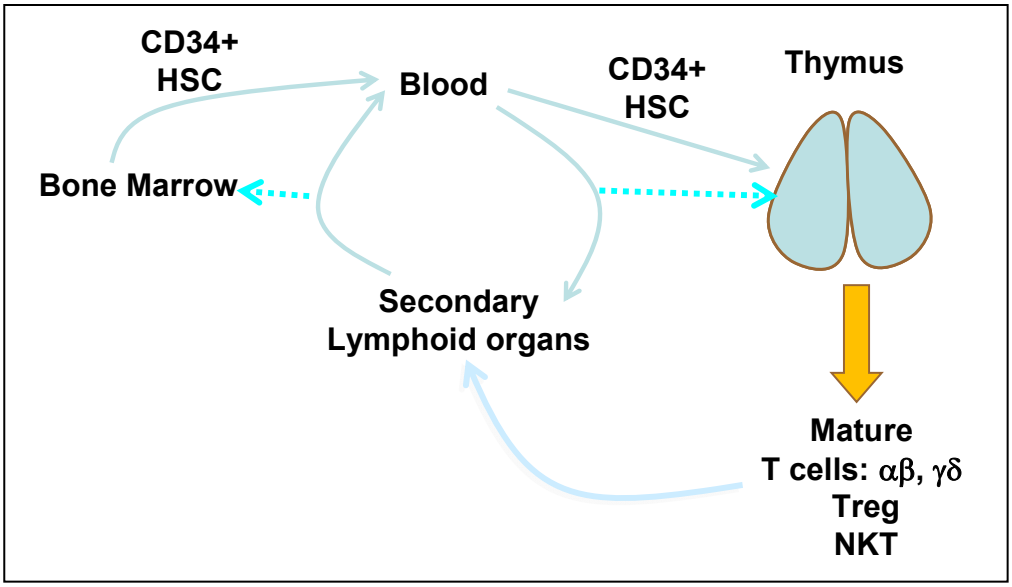
3. Thymocytes: The predominant lymphoid cells in the thymus. In postnatal animals, thymocytes are derived from progenitor cells of the bone marrow, the hematopoietic stem cells (HSC). After arriving in the thymus, bone marrow HSC progress through tightly regulated steps to develop into mature T cells. ***The thymus is responsible for the development of four functionally different T cells: CD4 T helper, CD8 T cytotoxic, regulatory T cells (Treg) and natural killer T cells (NKT).***

Flow cytomettric analysis is a powerful tool to identify thymocyte subsets: cells stained with anti-CD4 and CD8 antibodies are separated into 4 distinct populations: DP, double positive (CD4 and CD8 positive); DN, double negative (either CD4 or CD8 is expressed).



IV. Early Stages of Human T cell Development



The thymus is populated by blood born progenitor cells that are derived from the bone marrow hematopoietic stem cells (HSC). These cells express the unique cell surface marker CD34 and have the capacity to develop into T cells as well as B cells, dendritic cells, and NK cells. Because the migration of $CD34^{pos}$ cells is initiated at 7-8 week of gestation and is highly active in the

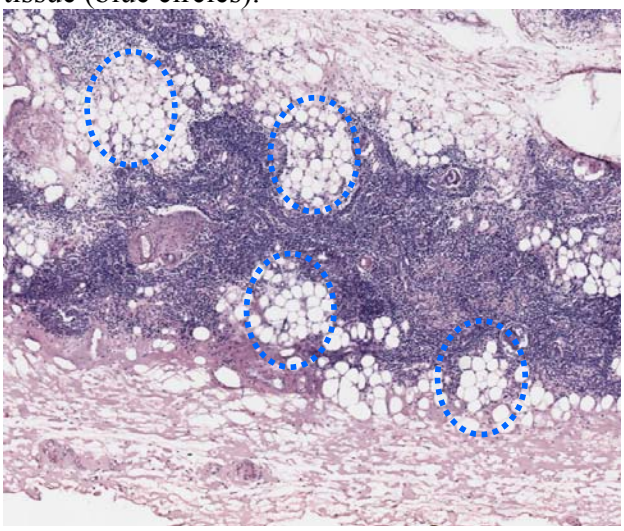
neonatal period, cord blood is good source for HSC $CD34^{pos}$ cells for transplant. Upon entry into the thymus, the lineage potential of the $CD34^{pos}$ cells is restricted to only the T lineage.

The production of T cells by the thymus is declines with age; below is the estimated daily thymic output of human T cells:

- 0-1 yr: $\geq 1.0 \times 10^9$
- 2-10 yr: 9×10^8
- 11-25 yr: 6×10^8
- 26-45 yr: 4×10^8
- $\geq$ 50 yr: 2×10^8

This age-associated decline in the production of T cells is responsible for the decline in immune response in the elderly.

Adult thymus: lost of cortical-medullar distinctions and presence of abundant adipose tissue (blue circles).

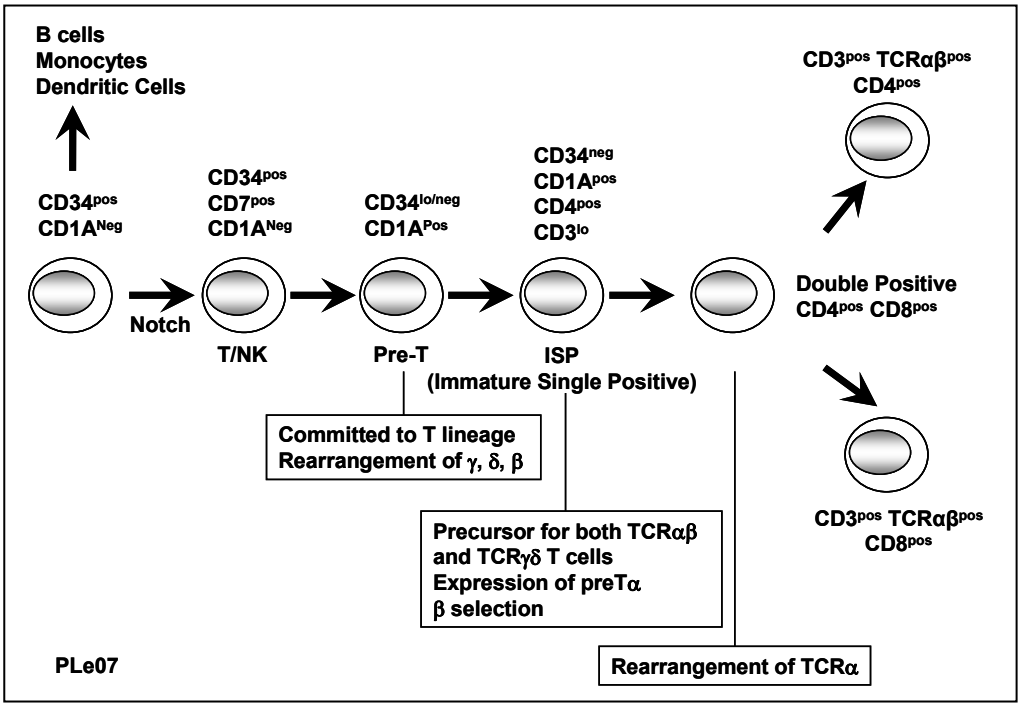


Host Defense
March 28, 2013

Development of T Lymphocytes
Phong T. Le, Ph.D.

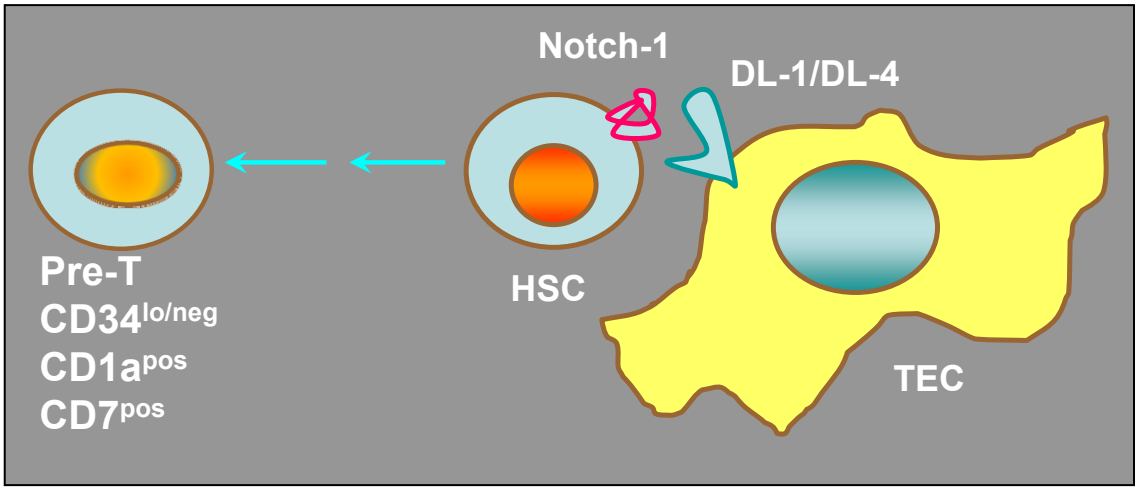
1. Four developmental events in T cells:

- T lineage commitment
- Proliferation and differentiation
- Selection: positive and negative
- Maturation



2. Identifiable stages of human T cell development

- The Notch receptor signal is essential for T cell lineage commitment of the $CD34^{pos}$ HSC. Signal through the notch receptor terminates the potential to commit to B and myeloid lineages (monocytes and DC). The cells have potential to become T or NK cells (T/NK). Persisting Notch signaling terminates NK development.



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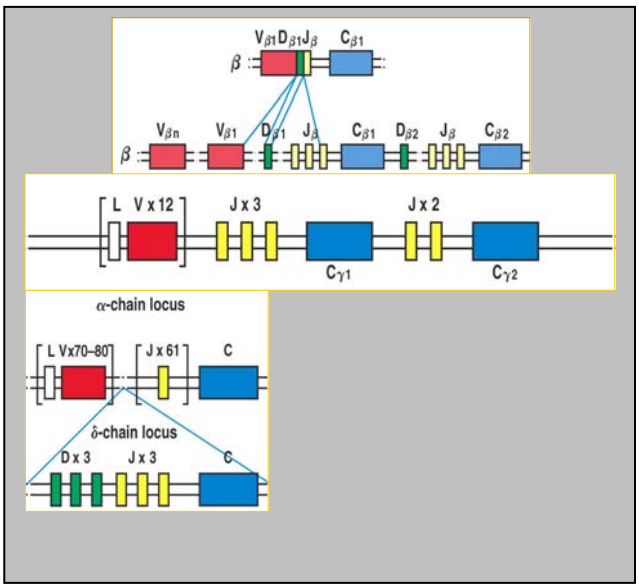
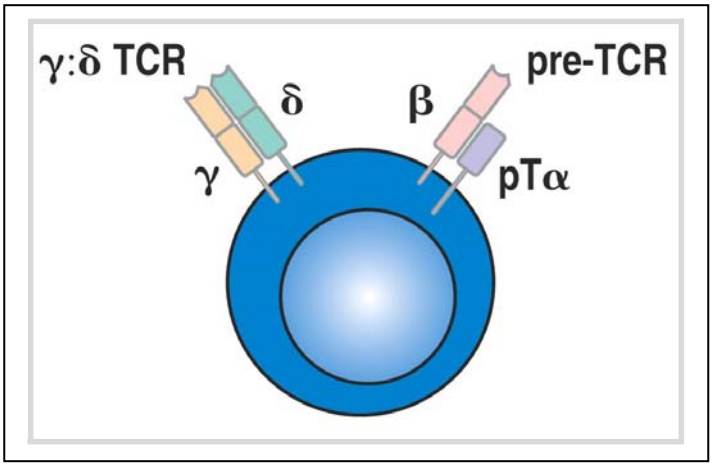
- Following Notch signal by Notch ligand DL4 and DL1, cells commit to T lineage, express CD1A and begin to rearrange TCRγ,δ and β genes. Cells at this stage, pre-T, can develop into either TCRγδ or TCRαβ T cells. Te rearrangement of TCR genes requires the recombinant activation gene -1 (RAG1) and RAG2. IL7 is also needed for this



developmental stage.

- In the next stage, cells express CD4 and become immature single positive (CD4ISP). These cells express the preTα (pTα); the expression of pTα also induces the expression of CD3. The pTα together with CD3 and TCRβ forms the preTCR complex. Signaling via pre-TCR generate a weak activation of the extracellular signal related kinase (ERK)



which terminate rearrangement of TCRγ and TCRδ genes.

- **β Selection:** The expression of the preTCR allows the ISP cells to undergo the β selection process. This process selects cells with productive and functional rearranged TCRβ genes signaling via the preTCR. Cells that do not from a functional preTCR die by apoptosis. Cells that survive the β selection step proliferate and expand. ***This enormous expansion of cells at this stage is responsible for generating the large number of thymocytes with TCRαβ in the thymus.*** Expression of CD3 is up-regulated at this stage.
- ISP cells that are selected progress to express CD8 and thus are termed CD4 CD8 double positive cells (DP); these cells begin to rearrange TCRα genes. Because the TCRδ locus

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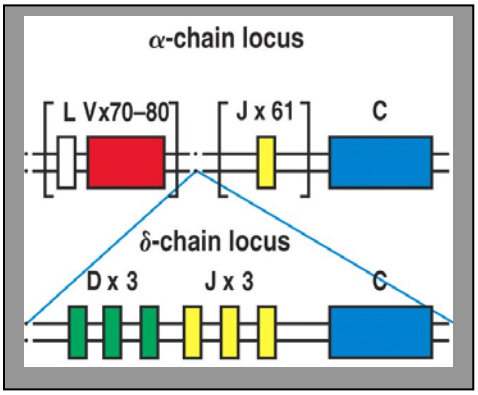
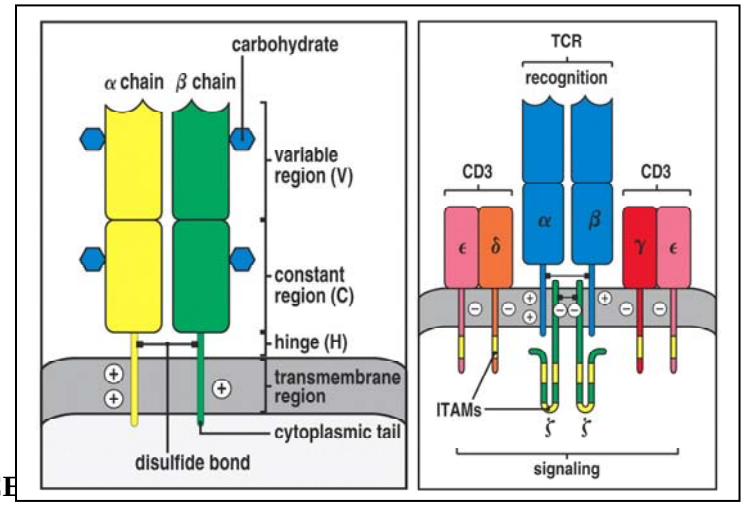
is within the TCRα genes, rearrangement of the TCR Vα genes will delete the δ locus. There is no allelic exclusion in TCRVα gene rearrangements. It is possible that there are two different rearranged Vα chains, each is associated with a common Vβ. Subsequent positive selection will ensure that each T cell only has a single functional specificity.

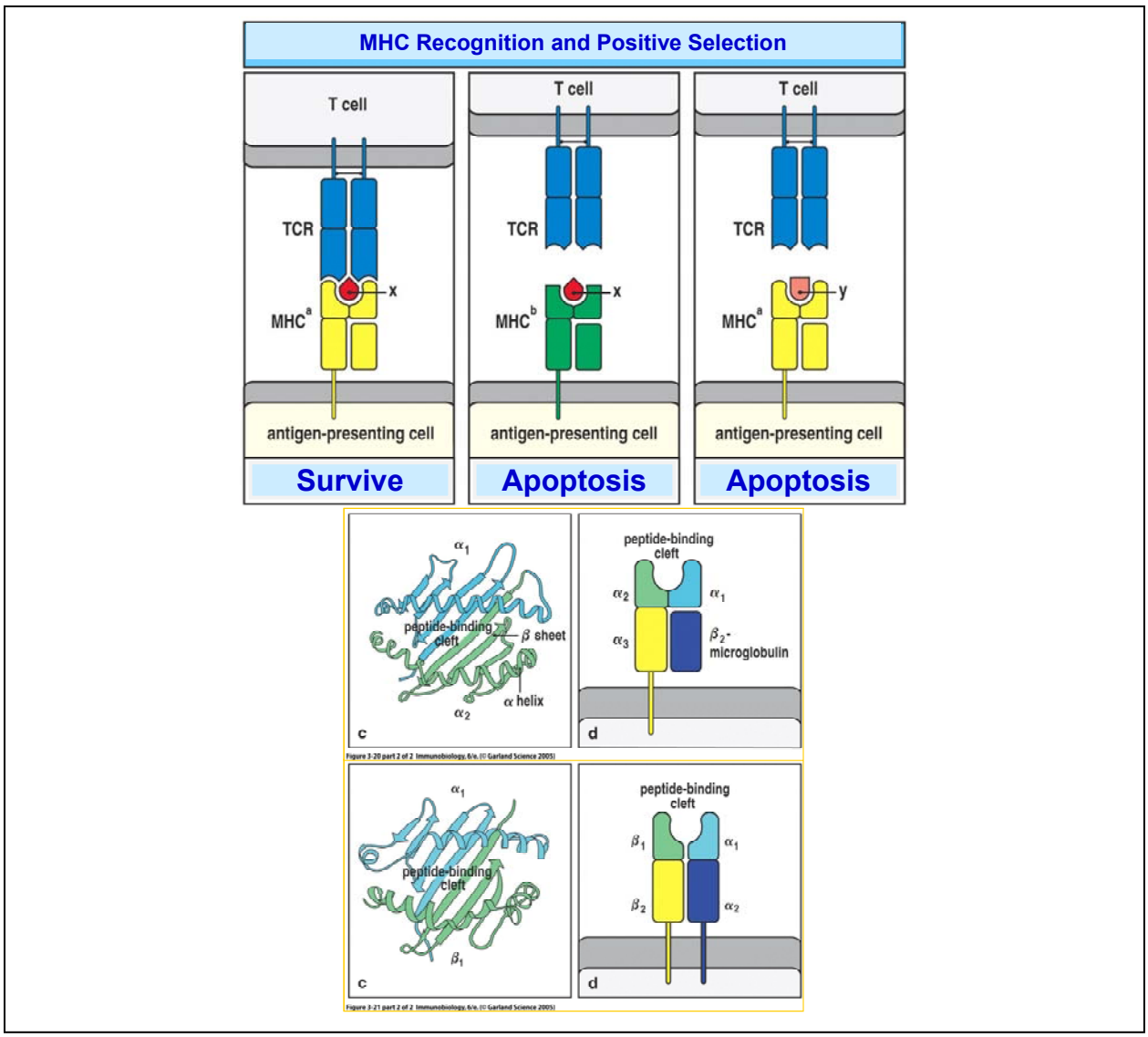
- Depend on the signal generated via the interaction of the co-receptor CD4 or CD8 surface molecules, DP cells down modulate one of the co-receptors and mature into either single CD4 or CD8 T cells.



3. Positive selection of TCRαβ T cells: Selection of T cells that recognize antigens presented by MHC molecules

- The DP thymocytes with functional TCRαβ/CD3 complex must recognize self-peptides in the MHC I and II complex presented by cortical TEC.
- The interaction of TCRαβ/CD3 complex with self peptides and MHC antigens must be of low affinity; DP cells with low affinity TCR for self peptides are rescued from apoptosis and proliferate (1 or 2 rounds of replications). These cells shut down expression of recombination-activating gene (RAG); no further rearrangement can take place if RAG proteins are not present.
- Cells express TCRαβ that do not recognize self peptides undergo apoptosis.
- Positive selection skews the selected TCRαβ repertoire toward self peptides and the potential of generating autoreactive T cell clones.
- Patients who receive bone marrow transplant generate T cells that recognize foreign antigens in the context of their own MHC antigens and not donor MHC, indicating that developing donor T cells occurs on TEC of the patient recipient.





4. Negative Selection: Deletion of mature T cells that bind strongly to MHC/antigens (autoreative T cells); negative selection results in the generation of central tolerance

- DP cells with TCRαβ/CD3 complex with high affinity for self-peptide and MHC are instructed to undergo apoptosis; this process is necessary to eliminate autoreactive T cells and to establish the central tolerance.
- Negative selection occurs predominantly in the cortical-medullary region where a high density of thymic DC cells is present. The DC cells are also responsible for phagocytosis of apoptotic cells.
- TEC also participate in clonal deletion of T cells that are reactive to organ specific antigens. The ***A***uto ***I***mmune ***R***egulator ***E***lement gene (*AIRE*) encodes a transcription factor that induces expression of a battery of peripheral-tissue antigens by thymic medullary epithelial cells; thus, AIRE promotes central tolerance of thymocytes by

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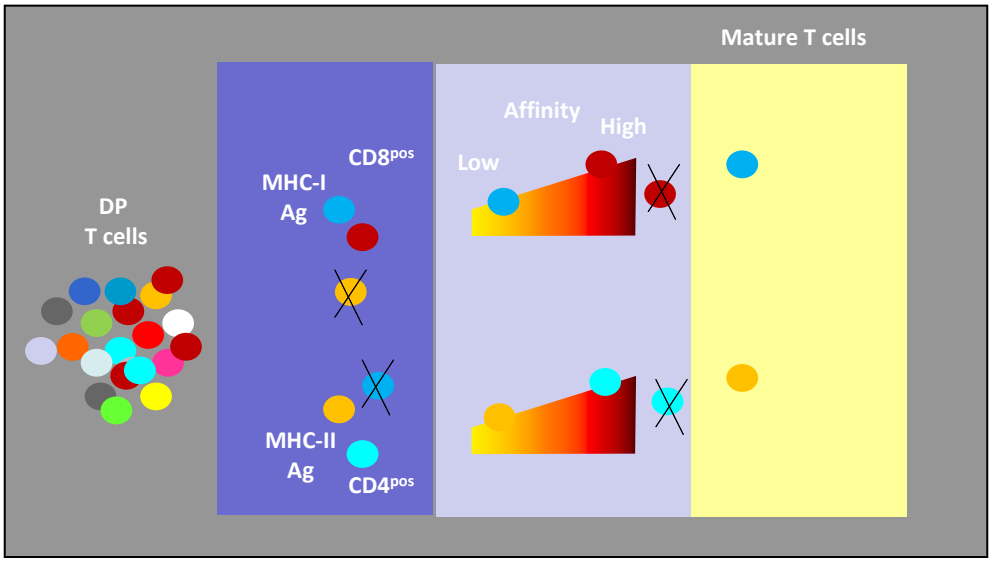
inducing negative selection, contributing to the prevention of organ specific autoimmunity.

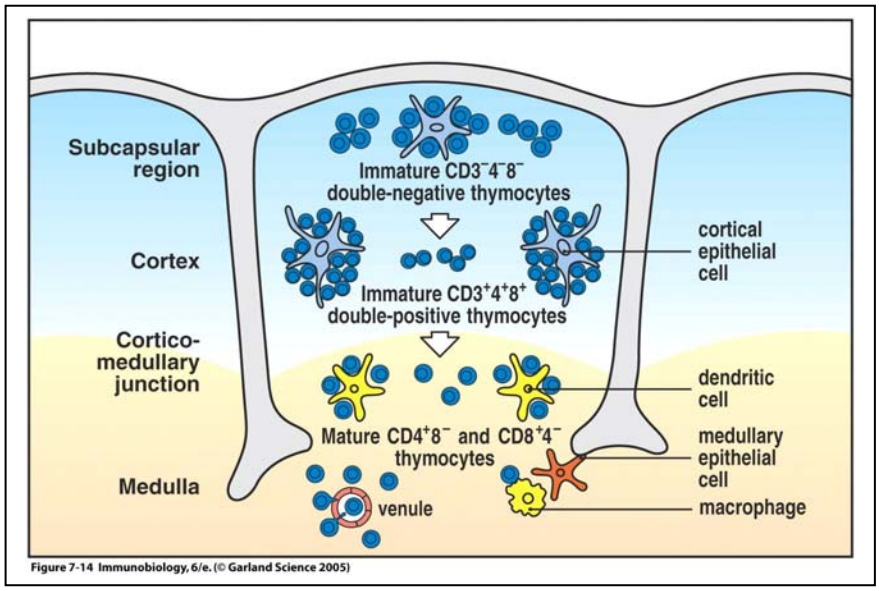
- Disease associated with mutations of AIRE: AIRE mutations are responsible for the development of autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) or Autoimmune polyendocrinopathy syndrome type 1 (APS1). The affected organs include: adrenal, thyroid, parathyroid, and pancreas.



5. Maturation toward CD4 or CD8 single positive

DP cells that survive both the positive and negative selection processes will commit to either single $CD4^{Pos}$ or $CD8^{Pos}$ cells

- Selection with CD4 co-receptor and MHC-II will generate mature CD4 cells.
- Selection with CD8 co-receptor and MHC-I will generate mature CD8 cells
- Mature CD4 and CD8 cells are predominantly resided in the medulla of the thymus.
- Mature CD4 and CD8 cells egress the thymus via blood vessels in the septa in the cortical-medullary junction.



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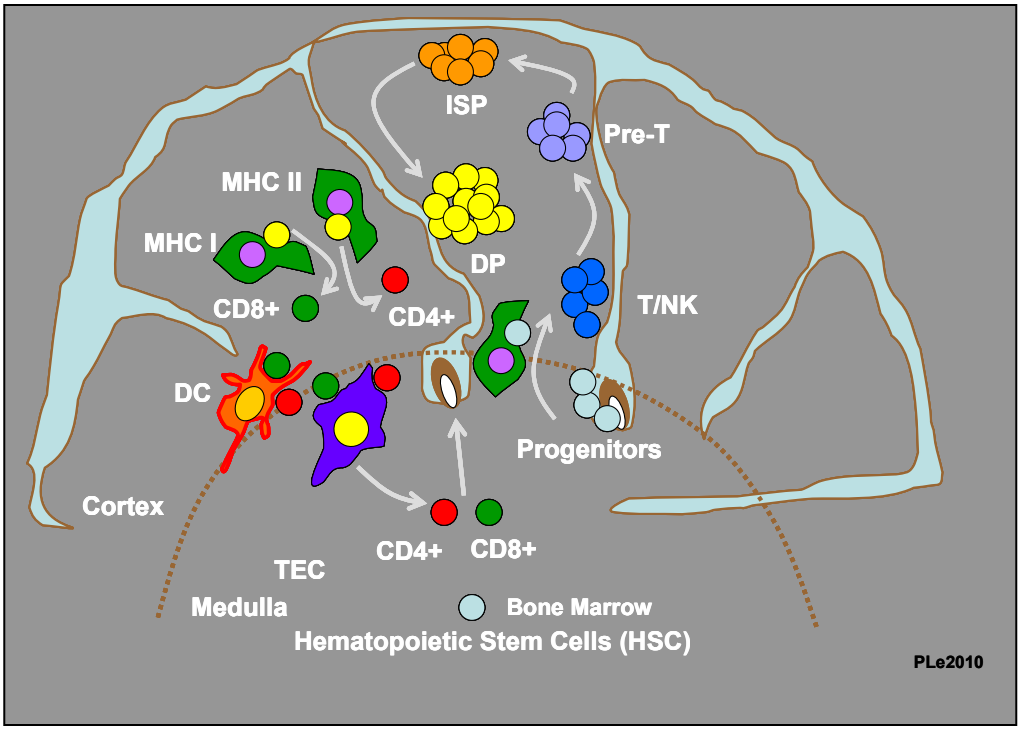
Overall process of T cell development in the human thymus



IV. TCR γδ T cells

The TCRγδ T cells develop from the ISP cells, the exact mechanism for the lineage choice between TCRαβ and TCRγδ is not well understood. It is generally accepted that the expression of pTα and signaling of this receptor skews the cells toward TCRαβ lineage.

TCRγδ cells:

- Only present at low level, they present 1-5% of spleen and lymph node T cells
- Are predominantly CD4 and CD8 negative
- The TCRγδ receptor bind antigens directly, no antigen presentation by MHC I or MHC-II is required
- TCRδγ repertoire is very narrow, there are two dominant TCRγδ T cells:

  a. Cells that express δ1 with various γ genes: these are the first γδ T cells that emerge from fetal thymus. They eventually populate epithelia tissues such as the intestine and skin. The TCRδ1 T cells recognize stressed cells and lipid antigens presented by CD1B or CD1C molecules.

  b. TCRγ9δ2: these are the majority of circulatory γδ T cells (80% of all γδ T cells)

Human CD1:

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- CD1 proteins transmembrane proteins that are distantly related to MHC molecules; however these proteins lack polymorphism.

    - There are four human CD1 proteins: CD1A, CD1B, CD1C and CD1D, each is encoded by different genes:
    - CD1B and CD1C are noncovalently associated with β2m
    - CD1B, CD1C and CD1D are known to bind to glycolipid antigens; CD1B and C particularly bind to bacterial glycolipid antigens.

TCRγδ functions:

- TCRδ1 cells: lyse stressed/ transformed epithelial cells
- TCRγ9δ2: recognize non-peptide pyrophosphomonoester antigens (phosphor antigens) found on mycobacterium and malaria parasite. These cells can also kill intra- and extracellular *M. tuberculosis,* produce IFNγ that affects cytotoxicity of NK and NKT cells and the generation of TH1 cells.

V. Natural Killer T cells (NKT)

1. Development of NKT cells:

- NKT cells develop in the thymus from the $CD4^{Pos}$ $CD8^{Pos}$ DP thymocytes.
- DP that recognize glycolipid antigens presented by CD1D positive cortical thymocytes develop into NKT cells
- NKT are either $CD4^{Pos}$ or $CD4^{Neg}CD8^{Neg}$
- NKT express both the NK maker CD56 and the T cell maker TCRαβ/CD3 complex
- Mature NKT egress the thymus and populate the liver, spleen, BM, and lymph nodes.
- Human NKT population constitutes approximately 0.02- 0.2% of the peripheral blood T cell compartment.

2. Functions:
- NKT cells rapidly produce both Th1 (IFN-γ) and Th2 cytokines (IL4, IL5, IL10) upon triggering; thus they play a role in immunoregulation.

VI. Regulatory T cells (Treg)

1. Discovery of Treg: first discovered in mice based on the following observations:
- Mice subjected to thymectomy between day 2 and day 4 post natal develop organ-specific autoimmune diseases
- The autoimmune diseases can be prevented by infusion of syngeneic T cells obtained from adult thymus or spleen
- It was later identified that the $CD4^{Pos}$ $CD25^{Pos}$ T cells are capable of suppressing the autoreactive T cells.

From these observations, it was concluded that the thymus generate $CD4^{Pos}$ $CD25^{pos}$ suppressor cells with specificity for autoreactive T cells that may escape negative selection during the development of T cells. This mode of suppression of autoreactivity is referred to as

dominant tolerance in contrast with central tolerance in which the autoreactive T cells are deleted.

2. Treg development in humans

- Develop in the thymus from $CD3^{Pos}$ $CD4^{Pos}$ T cells as early as 14 weeks of gestational age.
- Treg represent 5-10% of mature $CD4^{pos}$ thymocytes and 10% of peripheral blood $CD4^{Pos}$T cells.

3. Treg that develop in the periphery:

- Treg can be generated from peripheral mature $CD4^{pos}$ T cells by TGF-β
- T regulatory type 1 (Tr1)
- T helper 3 (Th3)

4. Treg and human diseases

Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX) is a clinical syndrome that presents with multisystem autoimmune disease. Clinically, patients with IPEX manifests most commonly with diarrhea, insulin-dependent diabetes mellitus, thyroid disorders, and eczema. FOXP3, the gene responsible for IPEX, maps to chromosome Xp11.23-Xq13.3 and encodes a transcription factor. Because patients with IPEX lack Treg, it was determined that expression of FOXP3 in $CD4^{Pos}$ T cells is required for the development of Treg in the thymus.

T Cell Development

Over One Hundred Years Ago……

The Source of Leukocytes and the True Function of the Thymus
By J. Beard D. Sc.

University Lecturer in Comparative Embryology, Edinburgh

" ….The thymus is the parent source of all the lymphoid structures of the body."

"… for just as the Anglo-Saxon stock has make its way from its original home into all part of the world, and has there set up colonies for itself and for its increase, so the original leukocytes, starting from its birth place and home in the thymus, have penetrated into almost very part of the body and have there created new centers for growth, for increase, and for useful work for themselves and for the body."

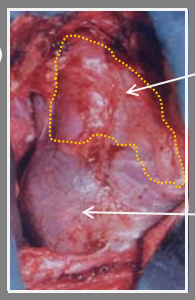
Anatomischer Anzeiger, JENA 18: 561-573, 1901

The Pediatric Human Thymus

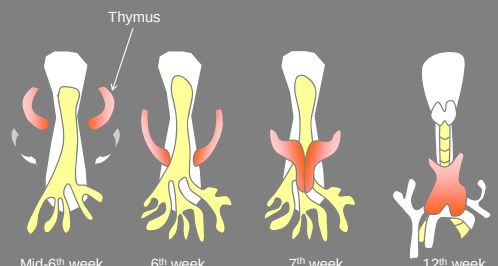
Hematopoietic stem cells (HSC) arrive from the bone marrow

In the thymus, HSC develop and mature into:

- T helper (CD4)
- T cytotoxic (CD8)
- Natural killer T (NKT)
- T regulatory (T_{reg})



Development of the Thymus and Generation of T Cells in Utero



Mid-6th week

6th week

7th week
Colonization by Hematopoietic stem cells

12th week
- Begin to produce T cells at 12-13th week
- Mature T cells egress the thymus at 13-14th week

T Cells are Detected in the Thymus During Embryonic Development

- Thymus glands are colonized by hematopoietic stem cells at 7-8 week of gestation
- Mature T cells are detectable in the thymus between 12-13th week of gestation
- Mature T cells egress the thymus at the end of 13th week of gestation.
- Peripheral T cell pool and its repertoire is established before birth
- Thymectomy afterbirth does not cause immediate immune deficiency

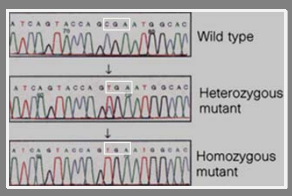
Genetic Evidence for The Thymus as The Organ for Thymopoiesis

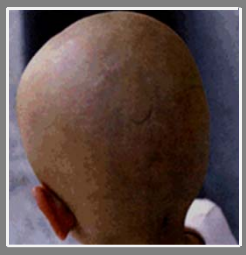
1. DiGeorge Syndrome or Velo-Cardio-Facial Syndrome (VCFS)

- Large deletion in 22q11 locus
- Hypoparathyroidism
- Heart defects
- Athymia: T cells are rarely detectable
- Thymus implants give rise to higher T cell number and restore immune response

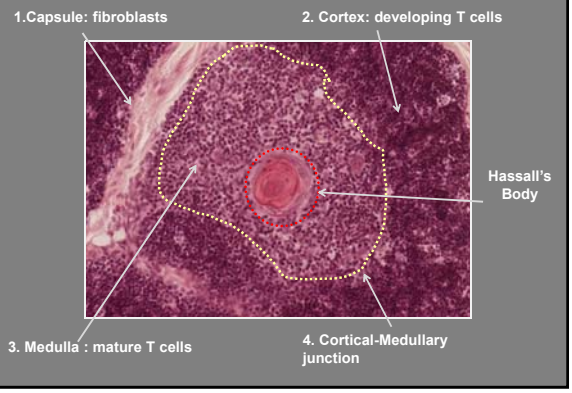
Genetic Evidence for The Thymus as The Organ for Thymopoiesis

2. Mutation in *FOXN1* gene
   - CGA to TGA: stop codon
   - No hair
   - No thymus

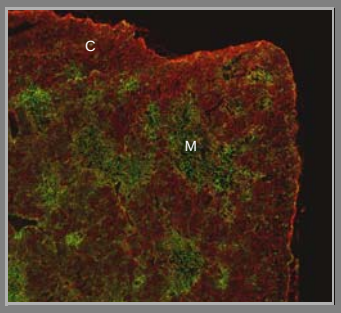




Thymus Histology



Thymus Histology : Epithelial Net work



Thymus: Cellular Composition

1. Thymic Stroma
   a. Fibroblasts: capsule, septa with blood vessels
   b. Epithelial cells: cortical, medullary and Hassall's body
2. Macrophages, Dendritic Cells
3. Lymphoid compartment: hematopoietic stem cells, thymocytes, mature T cells, natural killer T cells (NKT), regulatory T cells (T_{reg})

Thymic Epithelial Cells

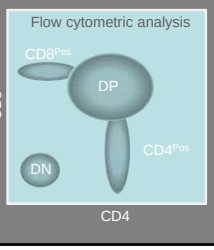
1. All TEC are derived from endoderm; expression of *FOXN1* is essential for functional maturation of TEC
2. Production of Cytokies:
   - IL7, SCF, IL1, IL6, IL15, Thymic Stroma Lymphopoietin (TSLP)
3. Cell surface ligand
   - Delta-Like 1, 4: ligand for Notch receptor
4. Expression of Major histocompatibility complex (MHC)
   - MHC I (HLA-A,B,C) and MHC II (HLA-DR)
5. Expression of peripheral tissue antigens: insulin

Macrophages, Dendritic Cells

- Derived from bone marrow
- Scattered in the thymic cortex and medulla
- Concentrated in the cortico-medullary junction
- Antigen presentation and phagocytosis of apoptotic thymocytes
- Deletion af autoreactive T cells : negative selection

Thymocytes and Their Progenitors

- Hematopoietic Stem Cells: $CD34^{Pos}$, <0.01%
- 80% of thymocytes are positive for the T cell markers CD4 and CD8; they are the immature double positive thymocytes (DP)
- Mature T cells
  - $CD4^{Pos}$: 10%
  - $CD8^{Pos}$: 5%
- Immature $CD4^{Neg}CD8^{Neg:}$ 5%



Daily Production of T Cells by the Thymus is Reduced with Age

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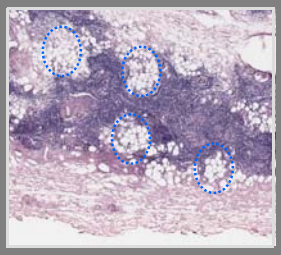
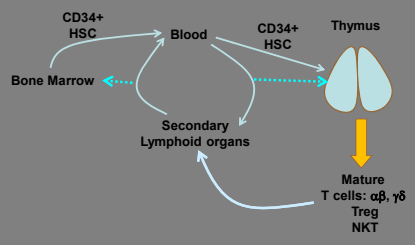
- 2-10 yr : 9×10^8
- 11-25 yr : 6×10^8
- 26-45 yr : 4×10^8
- ≥ 50 yr : 2×10^8



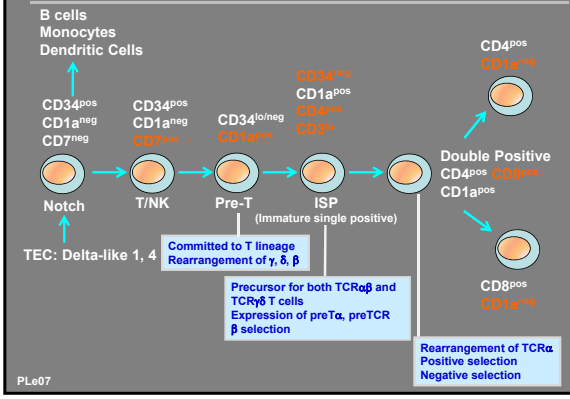
Circulation of HSC and Mature T Cells from The Thymus



Developmental Events in the Generation of Mature T cells

- T lineage commitment: restricted of lineage choices
- Proliferation: Expansion of committed cells
- Differentiation: gaining of new surface markers
- Maturation: gaining of immune functions

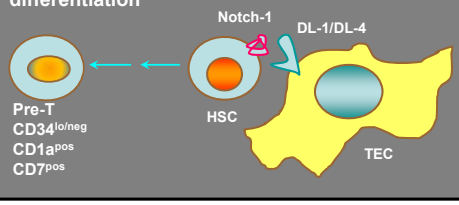
Stages in Human T Cell Development



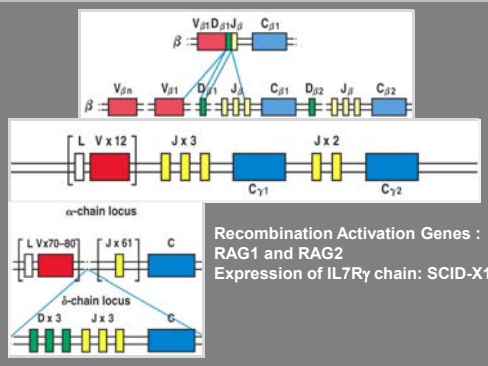
PLe07

T Lineage Commitment

- Notch receptor and Notch ligand Delta-Like 1 or 4 (DL-1, DL-4) interaction induces T lineage commitment and terminates lineage plasticity
- This is achieved by orchestrating expression of a set of transcription factors that are required for T cell differentiation



Pre-T: First Wave of TCR Gene Rearrangement

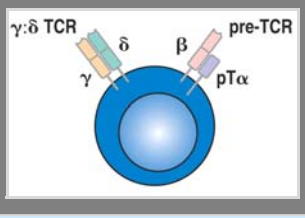


Recombination Activation Genes : RAG1 and RAG2
Expression of IL7Rγ chain: SCID-X1

Immature Single Positive Stage: β Selection

- Expression of the PreTCR complex:
  - Surrogate α chain (pTα), rearranged TCRβ and signal transduction molecules CD3
- β Selection: Selection for functional rearranged TCRβ
  - Terminates Vβ rearrangement by degrading RAG proteins
  - Induces rapid and vigorous proliferation
  - Increases expression of CD3 complex
- Signal for TCRαβ versus TCRγδ Development

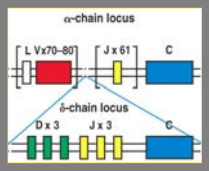
ISP Stage: TCRαβ versus TCR γδ



Combination of the rearranged TCRβ chain with pTα forms the pre-TCR. Signaling through this receptor induces a significant proliferation of ISP cells and bias the ISP toward TCRαβ lineage T cells.

The Double Positive Stage

- After β selection, ISP cells express CD8 and develop into $CD4^{Pos}$ $CD8^{Pos}$ double positive cells
- RAG expression is re-expressed and rearrangement of TCRVα genes are initiated. The rearrangement of Vα causes deletion of TCRδ locus



The Double Positive Stage

- There is no allelic exclusion in TCRVα rearrangement. It is possible that there are two different rearranged Vα chains, each is associated with a common Vβ. Subsequent positive selection will ensure that each T cell only has a single functional specificity, although two different α chains are expressed
- The DP cells now will first undergo positive and then negative selection

Functional TCR αβ

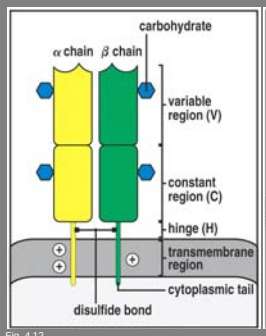


Fig. 4.12

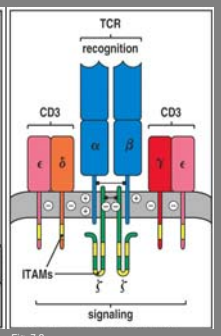


Fig. 7.8

Positive Selection of the DP TCRαβ T Cells

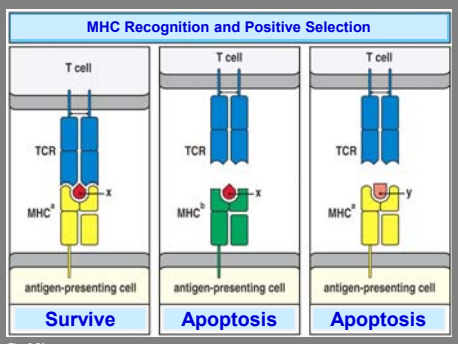


Fig. 6.21

Positive Selection Coordinates the Maturation of $CD4^{Pos}$ and $CD8^{Pos}$ T Cells

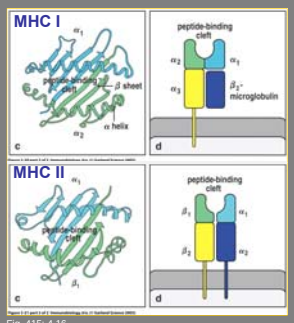


Fig. 4.15; 4.16

MHC expressed by cortical epithelial cells dictates down modulation of either the CD4 or CD8 co-receptors:

- MHC I/CD8: $CD8^{Pos}$
- MHC II/CD4: $CD4^{Pos}$

Bare Lymphocyte Syndrome (BLS):
Defect in MHC II expression; the development of $CD4^{Pos}$ T cells is affected

Positive Selection and Clinical Implications

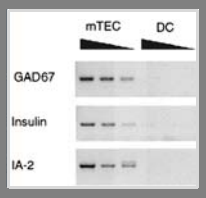
- Because the specificities of TCRs are selected on the basis of self MHC, the selected TCRαβ repertoire has the potential to generate auto-reactive T cell clones that may affect autoimmune diseases
- Patients received BM transplant will regenerate T cells that recognize foreign antigens in the context on their own MHC, not the donor MHC

Negative Selection: Mechanism for Establishing Central Tolerance

- When TCR on a DP cell binds antigen/MHC with high affinity, it is signaled to undergo apoptosis. This is the basis for intrathymic negative selection and the establishment of central tolerance
- Bone marrow-derived macrophages and dendritic cells in the cortical medullary junction are the prominent cell types that induce negative selection
- However, epithelial cells are also effective in inducing negative selection. This is relevant in patients who receive BM transplant, because macrophages and dendritic cells are of donor-derived.

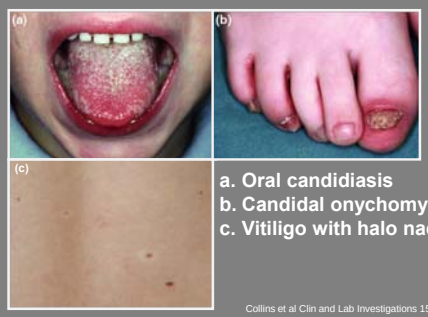
Negative Selection and Autoimmune Diseases

- Medullary thymic epithelial cells (mTEC) have been shown to express organ specific antigens that mirror peripheral self antigens
- An example of three specific pancreatic genes that are expressed by mTEC
- Expression of these genes are induced by the transcription factor AIRE
- These antigens are used to delete T cells with high affinity for these organ specific antigens



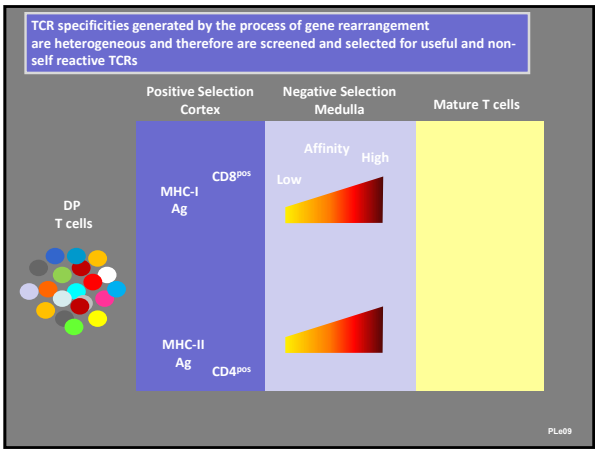
Derbinski et al Nature immunology 2: 1033, 2001

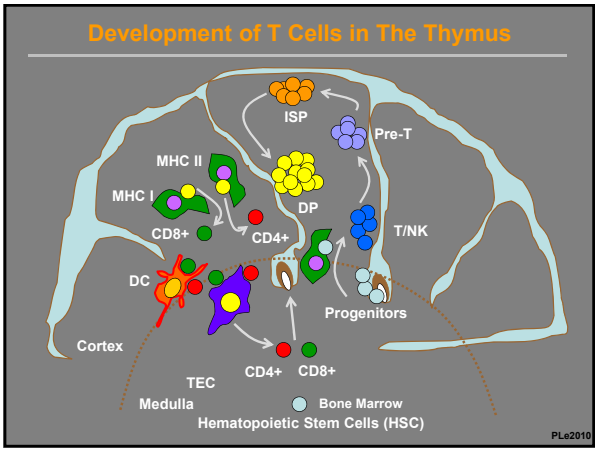
AIRE Mutation: Autoimmune Polyendocrinopathy Candiasis Ectodermal Dystrophy (APECED)

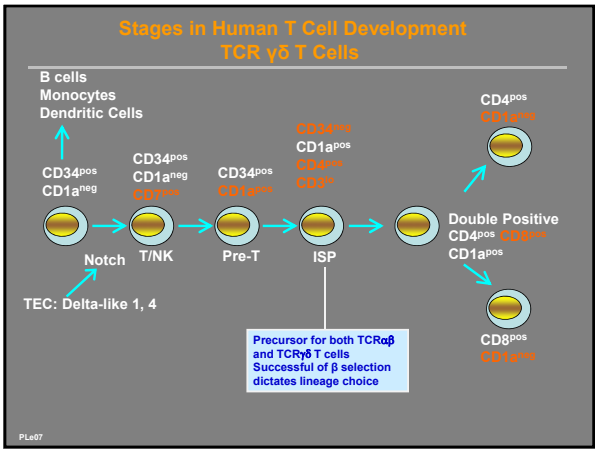


a. Oral candidiasis
b. Candidal onychomycosis
c. Vitiligo with halo naevi

Collins et al Clin and Lab Investigations 154: 1091, 2006


TCR specificities generated by the process of gene rearrangement are heterogeneous and therefore are screened and selected for useful and non-self reactive TCRs
Positive Selection Cortex
Negative Selection Medulla
Mature T cells
DP T cells
CD8pos
MHC-I Ag
MHC-II Ag
CD4pos
Affinity
High
Low
PLe09


Development of T Cells in The Thymus
ISP
Pre-T
MHC II
MHC I
DP
CD8+
CD4+
T/NK
DC
Progenitors
Cortex
CD4+
CD8+
TEC
Medulla
Bone Marrow
Hematopoietic Stem Cells (HSC)
PLe2010


Stages in Human T Cell Development
TCR γδ T Cells
B cells
Monocytes
Dendritic Cells
CD34pos
CD1aneg
CD34pos
CD1aneg
CD34neg
CD1apos
CD4pos
CD8pos
CD4pos
CD1apos
Notch
T/NK
Pre-T
ISP
Double Positive
CD4pos CD8pos
CD1apos
TEC: Delta-like 1, 4
CD8pos
Precursor for both TCRαβ and TCRγδ T cells
Successful of β selection dictates lineage choice
PLe07

TCR γδ T Cells

- Derived from ISP cells
- They are CD4 and CD8 negative
- TCRγδ bind antigens independent of MHC I or II; subsets of TCRγδ cells bind self lipid antigens presented by CD1b and CD1c molecules by stressed epithelial cells
- Restricted repertoire: only 3Vδ and 5Vγ
- Two major type of TCRγδ T cells based on their use of γ or δ chains
  - δ1 cells: mucosal tissue, associated with epithelial cells
  - TCRγ9δ2: majority of circulatory TCRγδ cells

Human CD1

- Trans-membrane proteins that are distantly related to MHC molecules but lack polymorphism
- There are 4 human CD1proteins: CD1a, CD1b, CD1c and CD1d; each is encoded by a different gene
- CD1b and CD1c are non-covalently associated with β2m; particularly bind to bacterial glycolipid antigens
- CD1b, c and d are known to bind to glycolipid antigens

Functions of TCR γδ T Cells

- TCRδ1: Cytotoxic activity, kill stressed-epithelial cells
- TCRγ9δ2:
  - Anti-microbial immunosurveillance; recognize intracellular bacteria such as *M. leprae, M. tuberculosis*
  - Immune regulation: through production of cytokines such as IFN- γ therefore affects cytotoxicity of NK and NKT cells and the generation of Th1 cells.

Natural Killer T Cells (NKT)

1. Development:
   - Express both markers for T cells (TCRαβ) and NK cells (CD56)NK
   - DP $CD4^{Pos}CD8^{Pos}$ thymocytes that recognize CD1d/glycolypids expressed on cortical thymocytes develop into NKT
   - NKT are $CD4^{Pos}$ or $CD4^{Neg}CD8^{Neg}$
   - Populate: liver, spleen, BM and lymph nodes
   - Present at 0.02-0.2% in the peripheral T cell pool

Natural Killer T Cells (NKT)

2. Functions

   - Immunoregulation:

     TH1 cytokines: IFN-γ, IL2:
     - NK: increase cytotoxicity, IFN-γ
     - CD4: TH1 response
     - CD8: increase cytotoxicity
     - Macrophages: increase phagocytosis

     TH2 cytokines: IL4, IL10, IL13:
     - CD4 : TH2 response

   - Cytotoxicity

Regulatory T Cells (T_{reg})

Discovery of T_{reg} in mice

- Mice develop organ specific autoimmune diseases if thymus are removed between day 2 and day 4 after birth
- Infusion of syngeneic T cells from adult thymus or spleen prevents the development of autoimmune diseases in thymectomized mice
- Infusion of $CD4^{pos}$ $CD25^{pos}$ thymocytes or T cells also prevent the development of organ specific autoimmune diseases

Conclusion:

Thymus derived $CD4^{Pos}$ $CD25^{Pos}$ cells have suppressor activity against autoreactive T cells that may have escaped the negative selection process. Thus T_{reg} cells establish dominant tolerance

Development of Regulatory T Cells (T_{reg}) in the Human Thymus

- Developed in the thymus from CD3pos CD4pos T cells around 14th week of gestation
- The development of Treg requires TSLP (thymic stroma lymphopoietin) expressed by TEC of the Hassall's bodies
- Expression of the transcription factor FOXP3 in the CD3pos CD4pos cells is essential for the development of Treg cells
- Treg represent 5-10% of mature CD4pos thymocytes and 10% of blood CD4pos cells
- Increases in the levels of Treg have been found associated with certain types of cancer

Regulatory T Cells (T_{reg}) Developed in the Periphery

- **TGF-β induces T_{reg} from $CD4^{pos}$ T cells**
- **T regulatory type 1 (Tr1): IL10**
- **T helper 3 (Th3): oral tolerance induction**

Regulatory T Cells and Human Disease

- **Patients with mutation in the *FOXP3* gene (Xp11.23-Xq13.3) have no T_{reg}**
- **Show multisystem autoimmune disease**
- **Described as Immune Dysregulation, polyendocrinopathy, and X-linked inheritance (IPEX)**