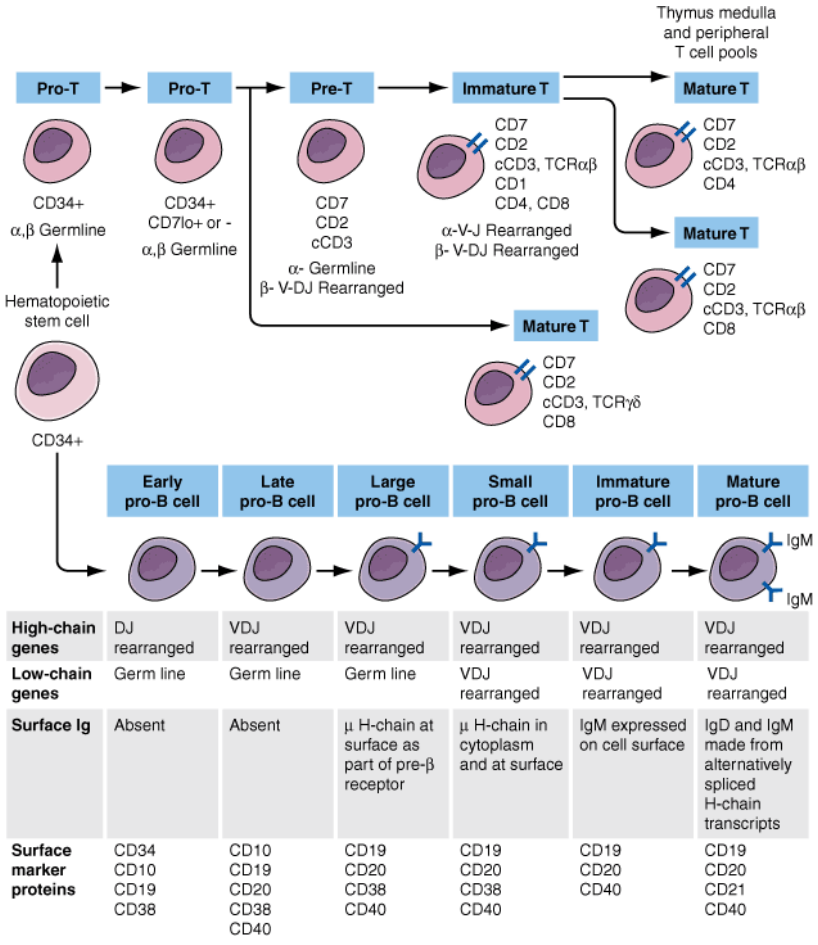
IMMUNOLOGY

T AND B CELL DEVELOPMENT

Kenneth Alonso, MD, FACP

T and B cell development



High-chain genes	DJ rearranged	VDJ rearranged	VDJ rearranged	VDJ rearranged	VDJ rearranged	VDJ rearranged
Low-chain genes	Germ line	Germ line	Germ line	VDJ rearranged	VDJ rearranged	VDJ rearranged
Surface Ig	Absent	Absent	μ H-chain at surface as part of pre-β receptor	μ H-chain in cytoplasm and at surface	IgM expressed on cell surface	IgD and IgM made from alternatively spliced H-chain transcripts
Surface marker proteins	CD34 CD10 CD19 CD38	CD10 CD19 CD20 CD38 CD40	CD19 CD20 CD38 CD40	CD19 CD20 CD38 CD40	CD19 CD20 CD40	CD19 CD20 CD21 CD40

Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

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Fig. 308-6 Accessed 07/01/2010

The classification into the various stages of B cell development is primarily defined by rearrangement of the immunoglobulin (Ig), heavy (H), and light (L) chain genes and by the absence or presence of specific surface markers. The classification of stages of T cell development is primarily defined by cell surface marker protein expression (sCD3, surface CD3 expression, cCD3, cytoplasmic CD3 expression; TCR, T cell receptor).

[Adapted from CA Janeway et al, (eds): Immunobiology. The Immune Systemic Health and Disease, 4th ed, New York, Garland, 1999; with permission.]

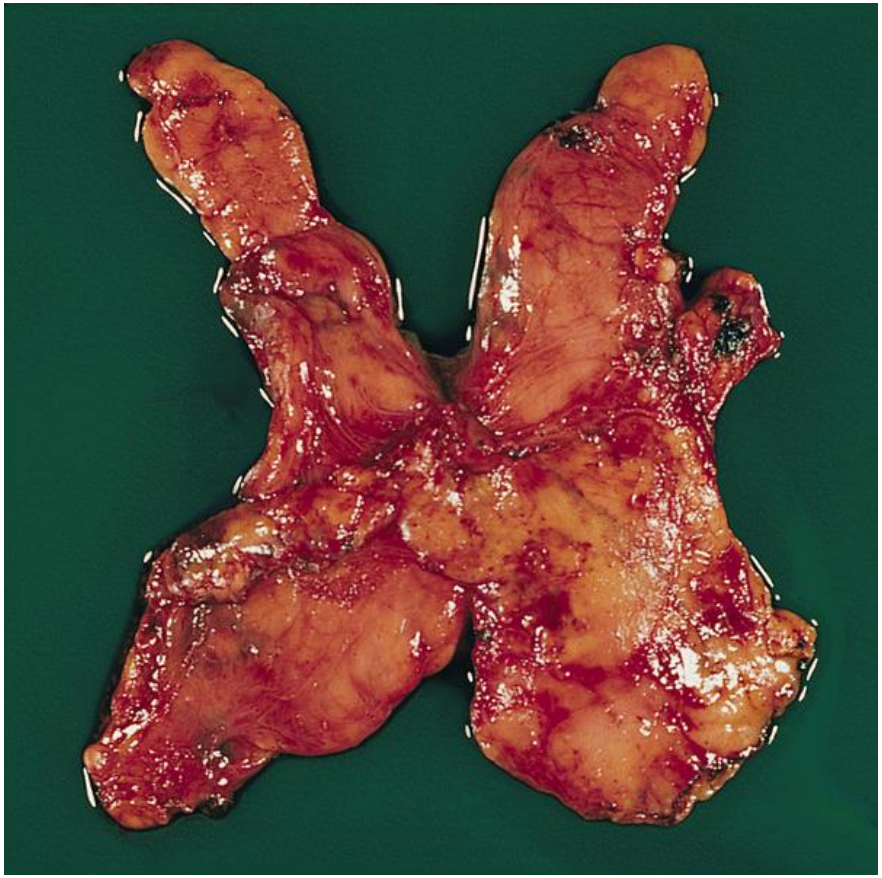
Major lymphoid organs

- Thymus
- Waldeyer’s ring (tonsils) and mucosal associated lymphoid tissue
- Lymph nodes
- Spleen
- Bone marrow

Thymus

- Develops from III pharyngeal pouch.
- Fully functional at birth.
- Loose and dense connective tissue form capsule.
- Trabeculae penetrate the tissue carrying vessels, nerves, and efferent lymph vessels.
- Lobules are formed by the trabeculae.
- Each lobule has an outer cortex with small lymphocytes and a lighter staining medulla with large lymphocytes.
- Lympho-epithelial organ largely replaced by adipose tissue by puberty.

Thymus

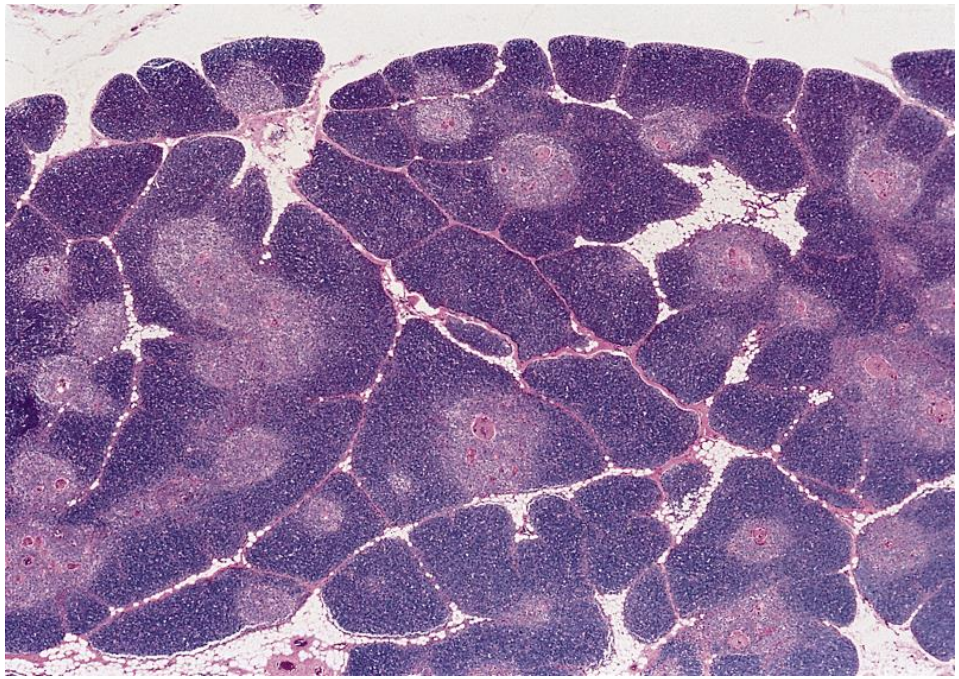


Infant thymus. Two separate lobes join at the lower poles. An adult thymus often shows an X- or H-shaped configuration. (Fig. 1-7 from Second Series, Fascicle 13.)

Fig. 1-6B

Shimosato, Y, Mukai, K., “Tumors of the mediastinum.” Atlas of Tumor Pathology, Third Series, Fascicle 21. Armed Forces Institute of Patholoy, Washington, D.C. 1997.

Thymus



Prominent lobulation by fibrofatty tissue septa is seen. Each lobule consists of dark cortex and pale medulla.

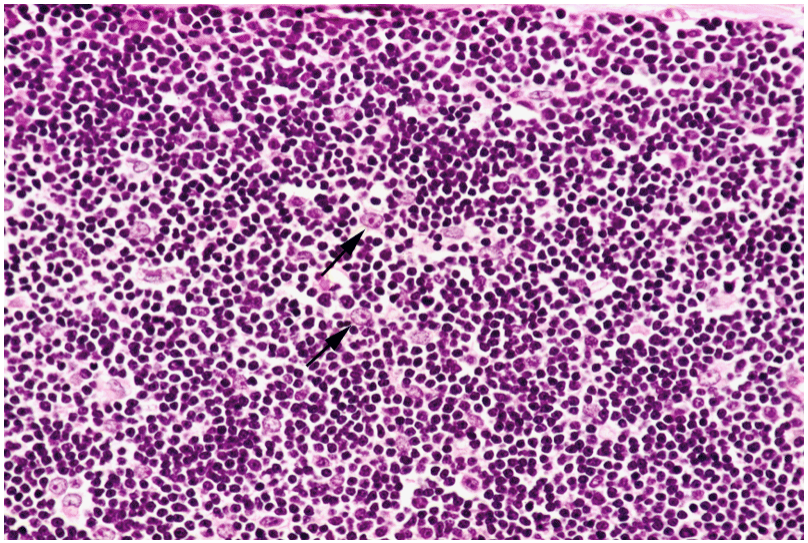
Fig. 1-8

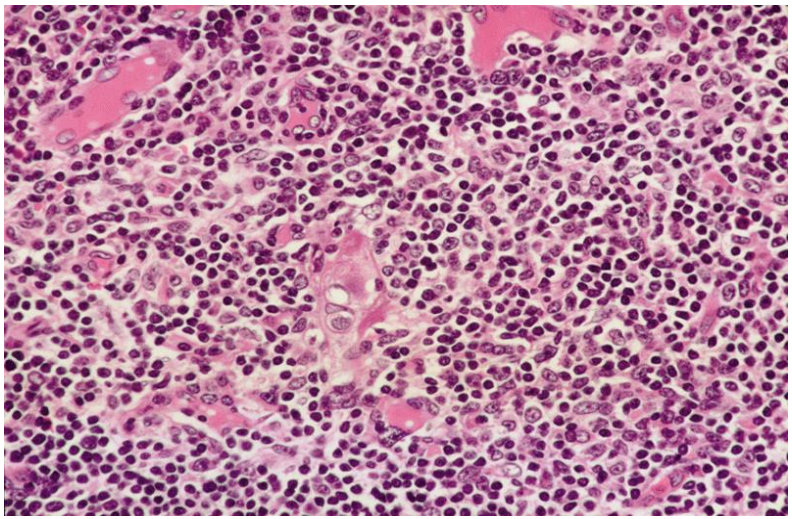
Shimosato, Y, Mukai, K., “Tumors of the mediastinum.” Atlas of Tumor Pathology, Third Series, Fascicle 21. Armed Forces Institute of Pathology, Washington, D.C. 1997.

Thymus

- **Epithelio-reticular cells:**
  - Features of both epithelial cells (intercellular junctions, intermediate filaments) and reticular cells. Form a network.
- **T cells (thymocytes):**
  - Between the elements of the network formed by epithelio-reticular cells
- **Macrophages**
  - Dispersed in the cortex
  - Phagocytise T cells that do not fulfill thymic education requirements.

Thymus





In the cortex (Top), scattered among lymphocytes are epithelial cells which possess round to oval clear nuclei and small but distinct nucleoli. In the medulla (Bottom), epithelial cells have spindle-shaped nuclei, coarse chromatin and interconnecting cell processes. A Hassall corpuscle is in the center.

Figs. 1-9AS and 1-9BS

Shimosato, Y, Mukai, K., “Tumors of the mediastinum.” Atlas of Tumor Pathology, Third Series, Fascicle 21. Armed Forces Institute of Pathology, Washington, D.C. 1997.

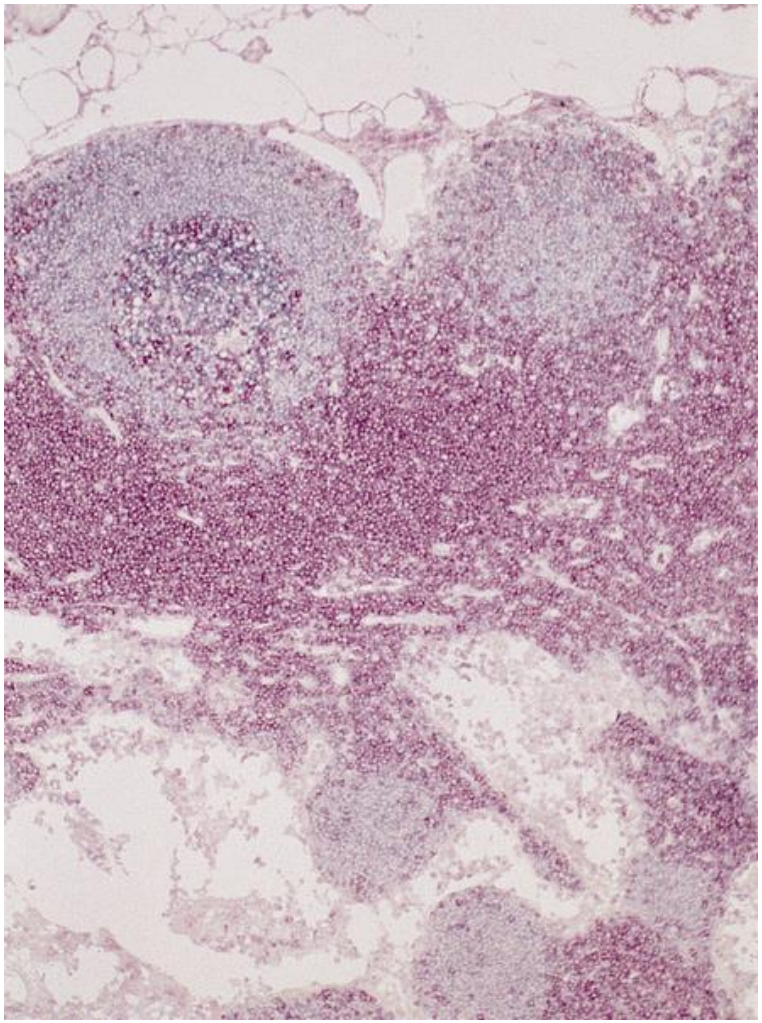
Lymph node

- Encapsulated lymphatic organ (1-2mm). Filters the lymph located along the lymphatic vessels.
- Capsule, trabeculae: dense connective tissue.
- Sinuses are subcapsular, trabecular, medullary (medulla in cords).
- Afferent lymphatic vessels enter at the convex surface.
- Efferent lymphatic vessels leave at the concave surface (hilum, the location of vessels, nerves, efferent lymph vessels).

Lymph node

- Superficial or nodular cortex consists of lymph nodules (follicles) that contain B cells and plasma cells.
- Deep cortex or paracortex (germinal center) contains T cells.

Normal lymph node

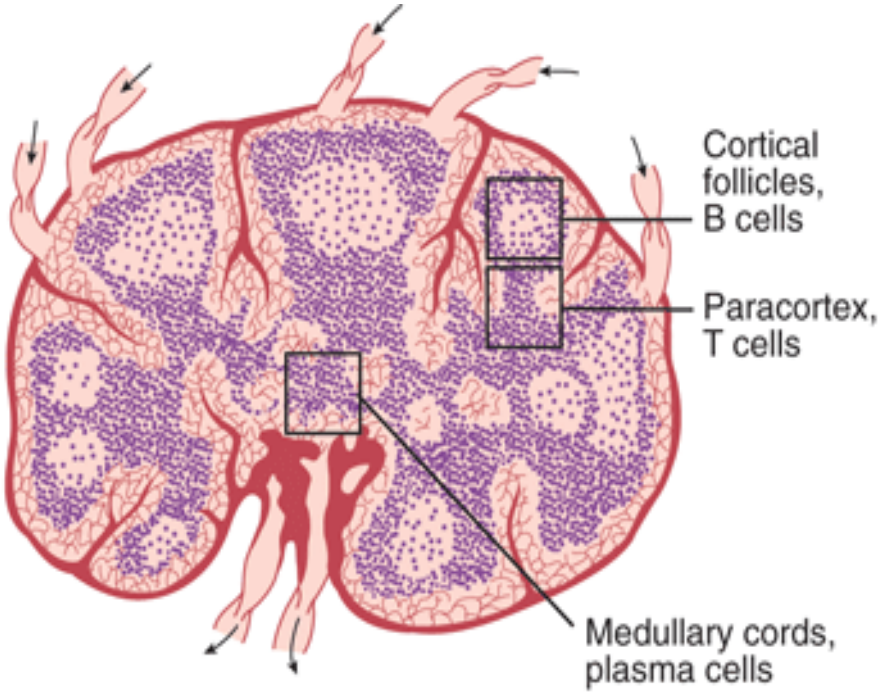


Primary and secondary follicles in the cortex lie adjacent to the paracortical T-cell zone. The subcapsular sinus connects to the medullary sinuses via trabecular sinuses. B cells predominate in the primary and secondary follicles as well as in the medullary cords (labeled blue with CD20) whereas T cells predominate in the paracortex (labeled red with CD43).

Fig. 2-4

Warnke, Roger A., Weiss, Lawrence M., Chan, John K. C., Cleary, Michael l., Dorfman, Ronald F., “Tumors of the lymph nodes and spleen,” Atlas of Tumor Pathology. Third series. Fascicle 14. Armed forces Institute of Pathology, Washington, DC. (1995).

Lymph node



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(After Chandrasoma. Reproduced with permission from McPhee SJ, Lingappa VR, Ganong WF (editors): *Pathophysiology of Disease,* 4th ed. McGraw-Hill, 2003.)

Fig. 3-2 Accessed 07/01/2010

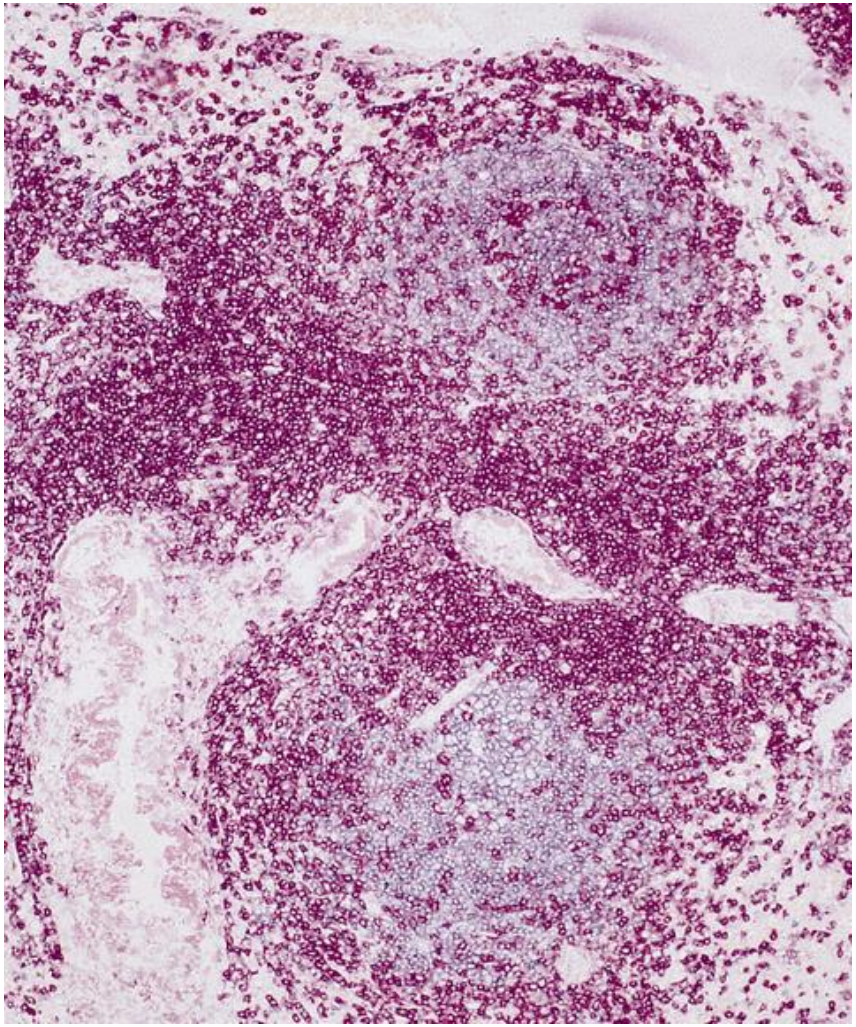
Mucosal associated lymphoid tissue

- B-lymphocytes with occasional plasma cells and eosinophils collect in submucosal tissue. They aggregate into lymphatic nodules with follicular centers.
- Gut, bronchus, tonsils are three principal sites for mucosal associated lymphoid tissue.

Spleen

- The spleen is the largest lymph organ.
- It is divided into **red pulp** and **white pulp.**
- T-cells are found in the peri-arteriolar lymphoid sheath in the red pulp. B-cells are found in the marginal zone around a germinal center in the white pulp. May form secondary follicles if stimulated.
- On the surface of the organ is a dense irregular connective tissue **capsule** containing myofibroblasts. From this capsule **trabeculae** project into the pulp of spleen dividing the spleen into lobes.

Normal spleen



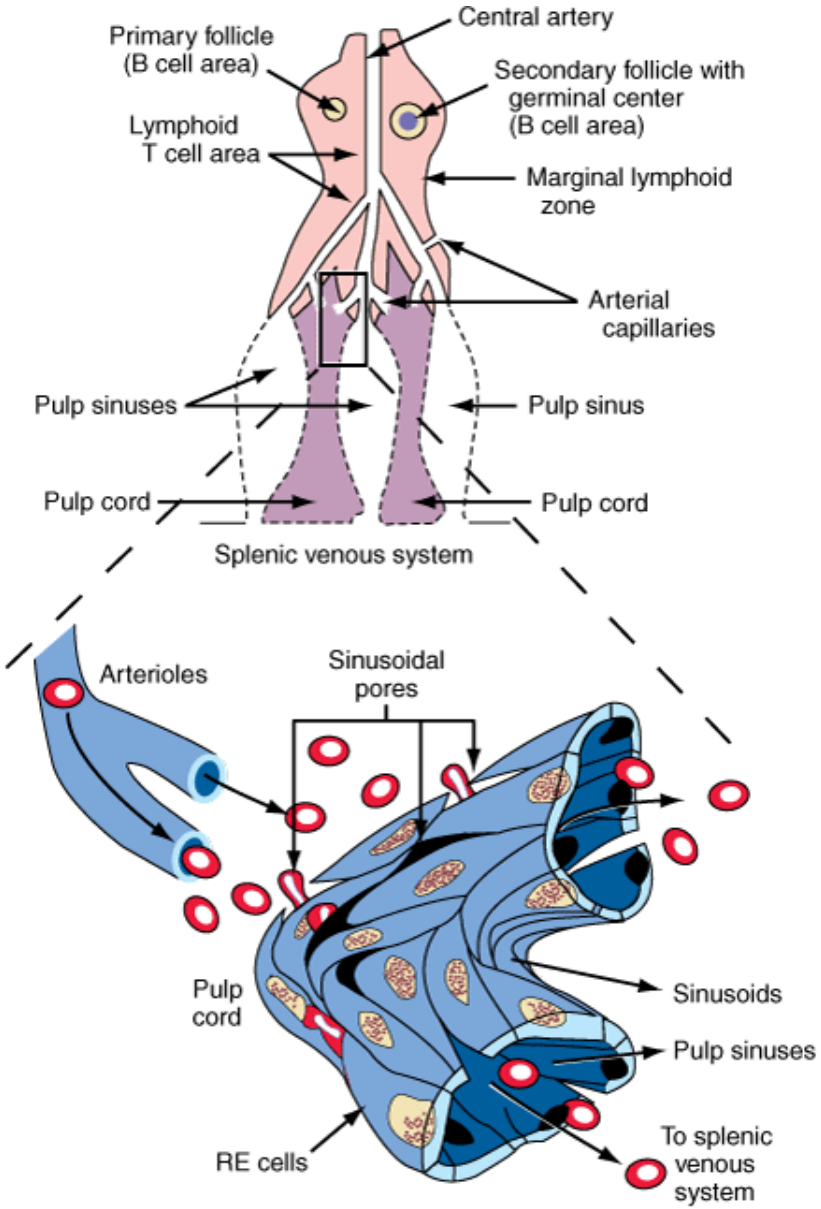
The T cells predominate in the periarteriolar lymphoid sheath (labeled red with CD43). The follicles, which tend to occur at arterial branch points, are labeled blue (CD20).

In the spleen, a central germinal center is surrounded by a thin mantle of small lymphocytes (pale staining) internal to a prominent marginal zone. Macrophages are preferentially found in the marginal zone and red pulp.

Fig. 2-16

Warnke, Roger A., Weiss, Lawrence M., Chan, John K. C., Cleary, Michael l., Dorfman, Ronald F., “Tumors of the lymph nodes and spleen,” Atlas of Tumor Pathology. Third series. Fascicle 14. Armed forces Institute of Pathology, Washington, DC. (1995).

Splenic circulation



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(Top portion of figure from CA Janeway et al: Immunobiology, 5th ed., New York, Garland, 2001; bottom portion of figure from RS Hillman, KA Ault: Hematology in Clinical Practice, 4th ed. New York, McGraw-Hill, 2005.)

Fig. 60-1 Accessed 07/01/2010

Lymphocyte traffic

- Lymphocytes (B or T cells) must be able to continuously circulate through the secondary lymphoid tissues in order to increase the chances that they will come in contact with antigen
- Once a lymphocyte recognizes antigen, within 24 hours of antigen localizing in the lymph node, antigen-specific lymphocytes are depleted from circulation, localize in the lymph node or spleen and become activated
- The flow of B and T cells through the secondary lymphoid tissues is directed by cell adhesion molecules (CAM's) and specialized endothelial cells in the high endothelial venules (HEV).

Transmigration

- **Rolling**
- Selectins on the lymphocyte bind CD34 on the vascular endothelium.
- **Attachment**
- Endothelial binding activates β_2 integrins.
- **Arrest and Adhesion**
- β_2 integrins change conformation and bind very strongly to intercellular adhesion molecules on the surface of the vascular endothelium.
- **Transendothelial migration**

Immune response

- Antigen enters the Peyers patches across specialized phagocytic cells (M cells).
- After activation, lymphocytes enter lymph, go through lymph node and thoracic duct, then pass from the blood back into the lamina propria and become IgA secreting plasma cells
- A large concentration of activated T cells with a CD4:CD8 ratio of 7:3, B cell blasts and IgA+ plasma cells are found in the lamina propria.
- Intraepithelial lymphocytes are principally T cells, but 10-40% are T-cell Receptor γδ cell that do not rely on MHC protein display.

Mediation of immunity

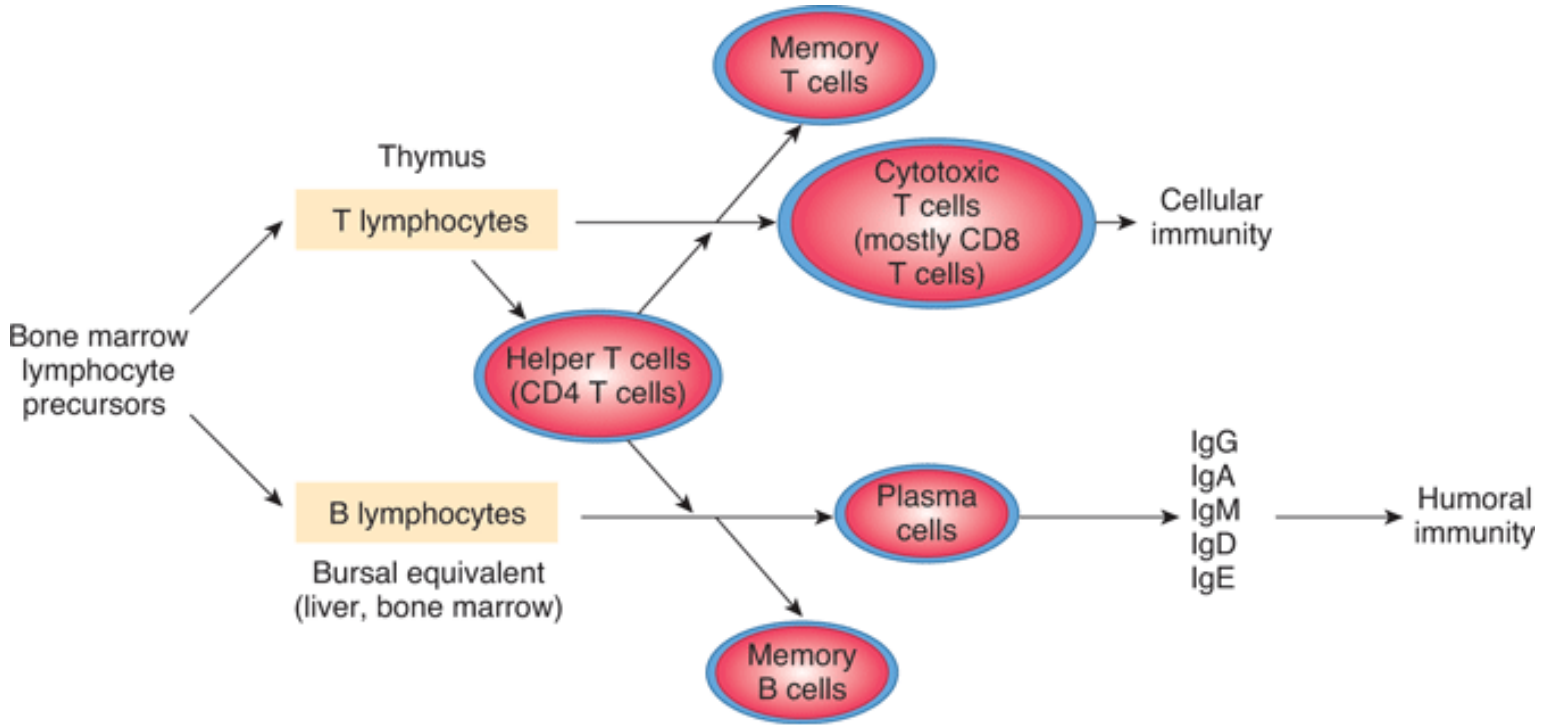

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Fig. 3-5 Accessed 07/01/2010

Acquired immune response

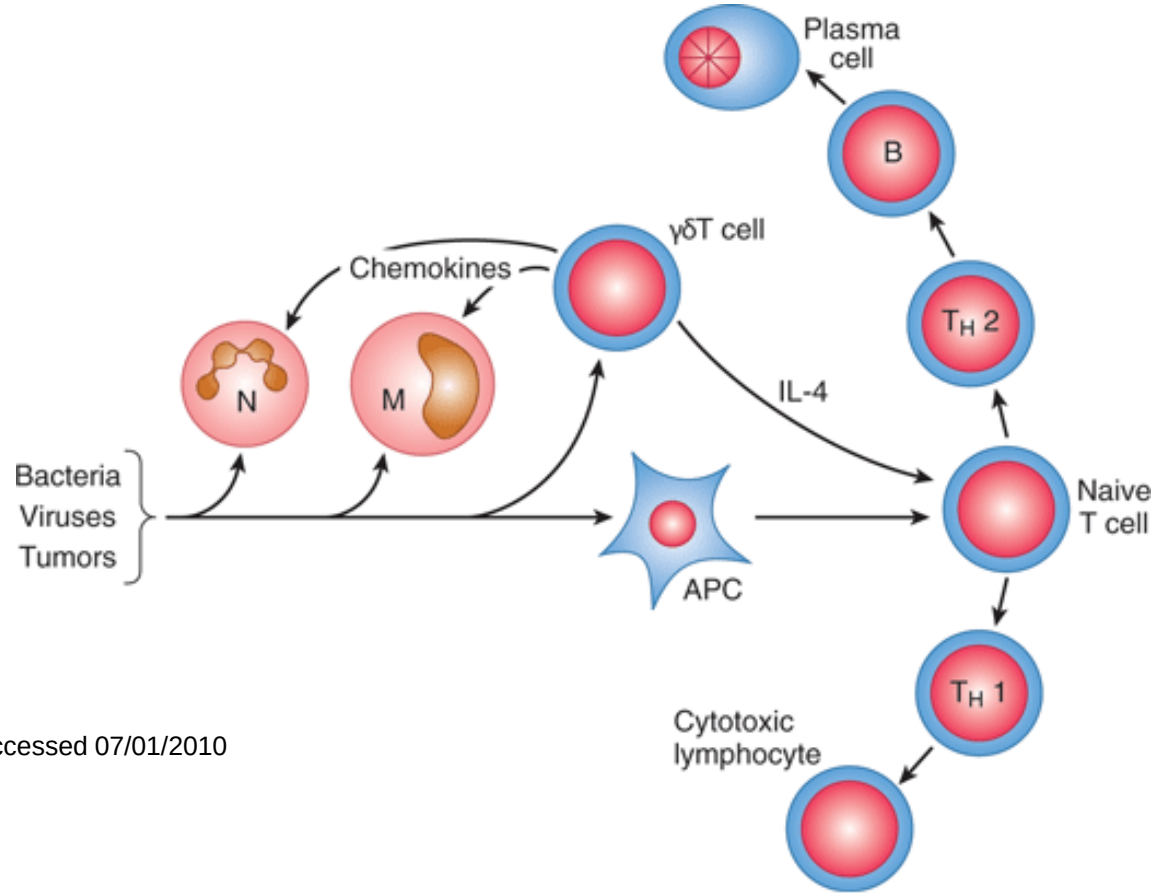

Fig. 3-3 Accessed 07/01/2010

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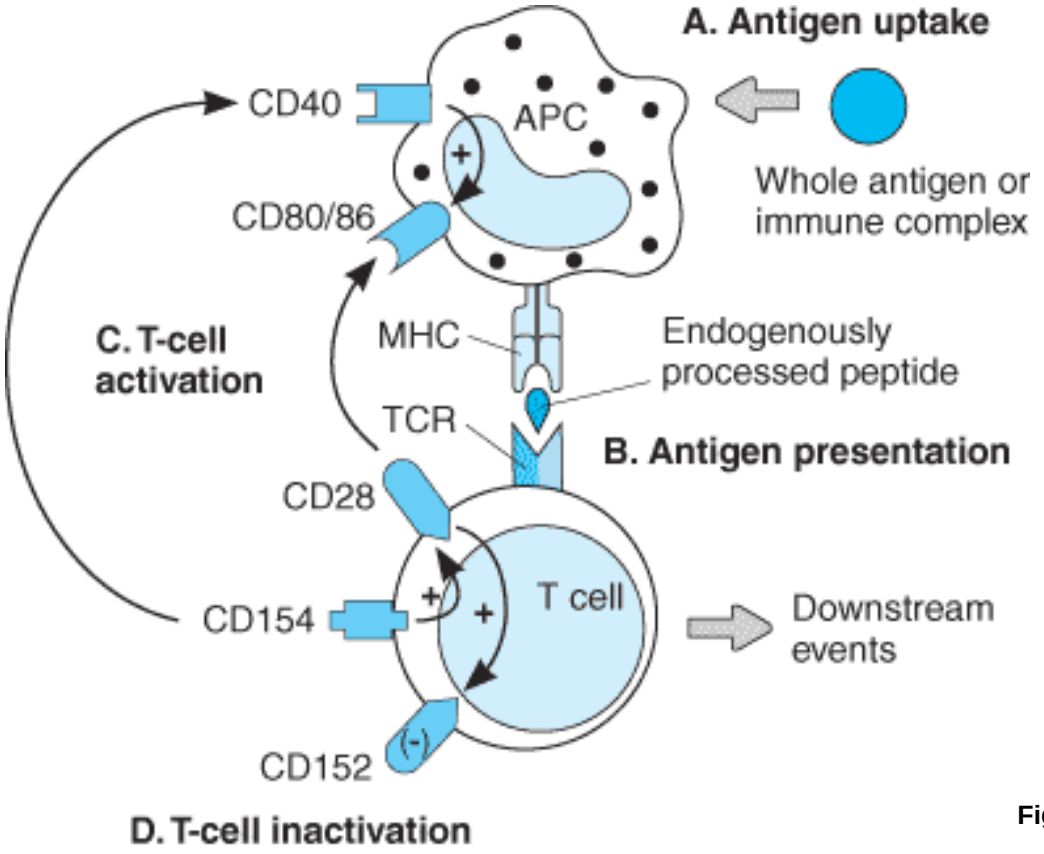
Antigen presentation (old model)

- According to the traditional model, T_H cells and T_C cells recognize antigen on the same APC. The APC-activated T_H cell produces interleukin-2 (IL-2), which contributes to the activation of T_C cells while in simultaneous interaction with the same APC.

T-cell antigen recognition



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Antigen presentation (new model)

- According to the proposed new model**,** APCs are allowed (“licensed”) to activate T_C cells by T_H or non-MHC molecules (lipopolysaccharides, IFN-γ, viruses). APCs first interact with T_H cells. The association of CD154 (CD40 L) on the T_H cell and CD40 on the APC allows the latter to activate T_C cells directly. Thus, once informed, APCs are capable of activating cytotoxic T cells without the need of simultaneous interaction with T_H cells.

Antigen presentation (new model)

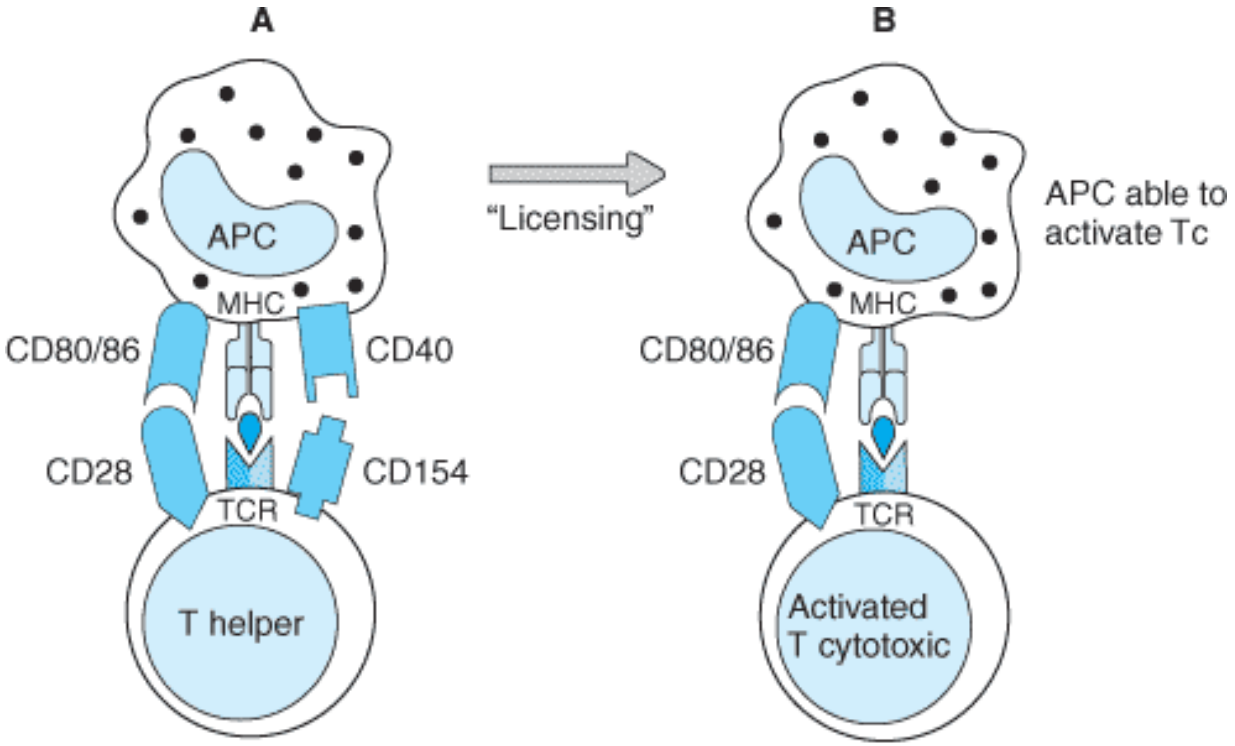

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Fig. 3-4 Accessed 07/01/2010

Mechanism of central T-cell tolerance

- Multipotential lymphoid stem cells from the bone marrow infiltrate the thymus in the embryo.
- Pre-T cells first rearrange their T-cell receptor. Unproductive (nonfunctional) rearrangements lead to apoptosis, while productive ones engage pre-T cells in self-antigen recognition.

T-cell receptor arrangement

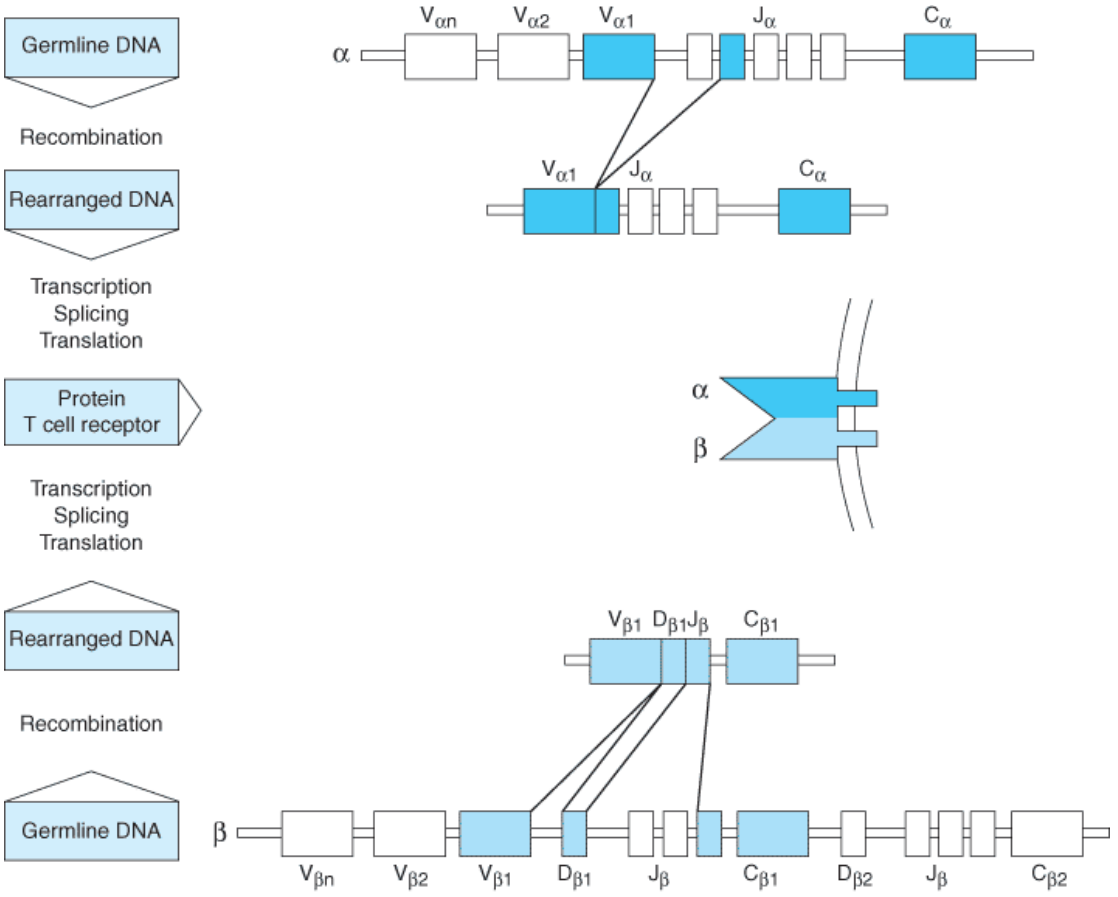

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Fig. 3-1 Accessed 07/01/2010

T-cell receptor

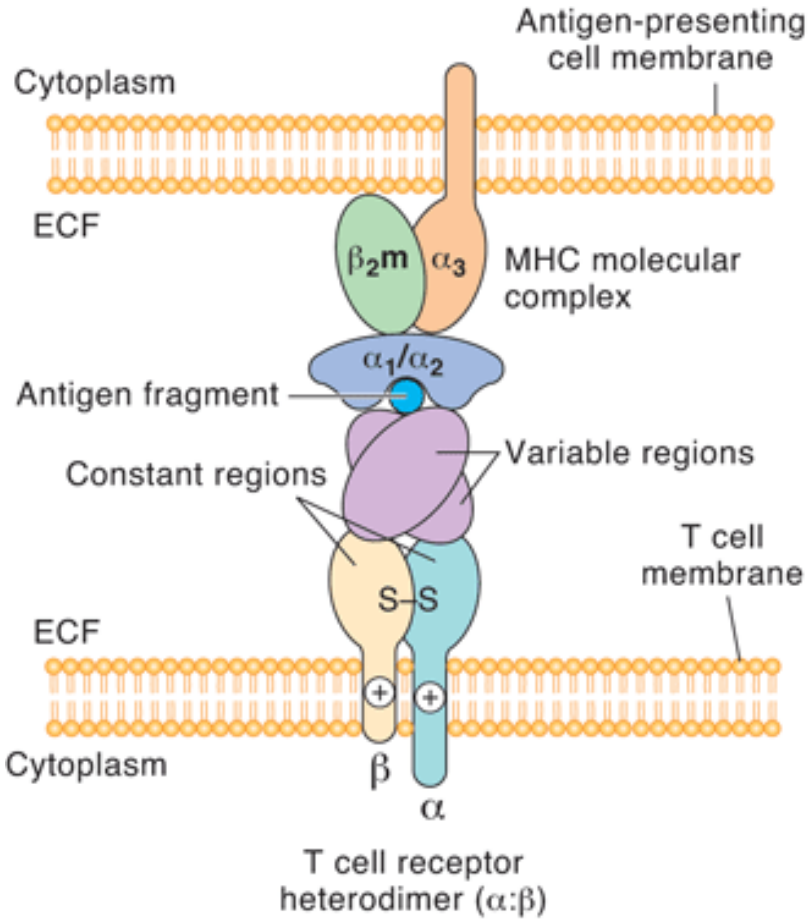

Fig. 3-7 Accessed 07/01/2010

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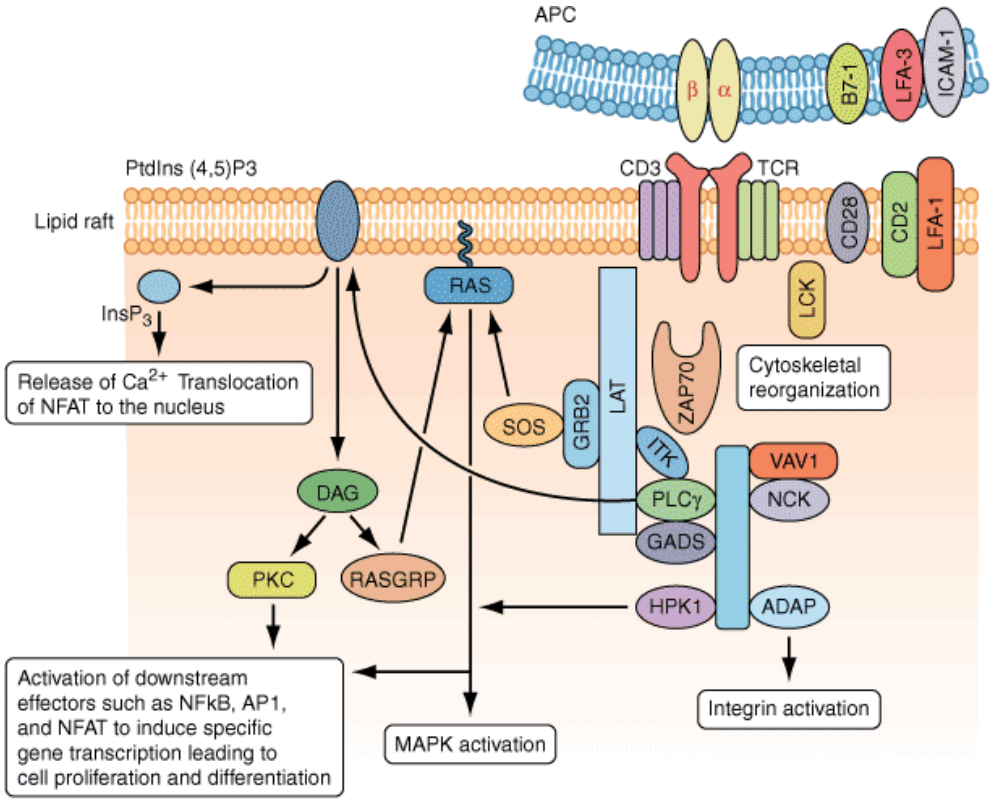
Signaling through the T cell receptor



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[Adapted from GA Koretzky, F Abtahaian, MA Silverman, SLP76 and SLP65: Complex regulation of signalling in lymphocytes and beyond. Nature 6(1):67–78, 2006; with permission.]

Fig. 308-7 Accessed 07/01/2010

Mechanism of central T-cell tolerance

- T cell selection is characterized by expression and deletion of surface antigens:
- Positive selection: cells are presented with self and foreign antigens by type II and III epithelio-reticular cells. If lymphocyte recognizes self-antigens with low affinity, it will survive. Cells that pass the selection enter the medulla.

Mechanism of central T-cell tolerance

- Negative selection: cells with high affinity to self-antigens or no affinity to self-antigens are eliminated (clonal deletion).
- AIRE protein stimulates expression (in thymus) of self-antigens ordinarily restricted in peripheral tissues.
- Surviving low-avidity cells reach the periphery as mature CD4 and CD8 cells.

Mechanisms of peripheral T-cell tolerance

- **Clonal deletion:** After encountering self-antigen in the context of self major histocompatibility complex (MHC) molecules and simultaneous delivery of a second signal (CD80/86-CD28) by antigen-presenting cells (APCs), autoreactive T cells become activated.
- These activated T cells express FAS molecules on their surface but are resistant to FAS ligand-mediated apoptosis because of the simultaneous expression of BCL-xL induced by CD28 ligation during activation.

Mechanisms of peripheral T-cell tolerance

- **Clonal deletion (continued):** Several days after activation, when BCL-xL presence has declined, CD4 cells become susceptible to FAS ligand-mediated apoptosis. Natural killer cells (NK-T) may then accomplish the task of eliminating these autoreactive T cells.
- **Anergy*:*** Anergy may be induced via CD80/86-CD152 interaction 48–72 hours following activation or may result from the lack of a second co-stimulatory signal from APCs presenting self antigen (nonprofessional APCs).

Mechanisms of peripheral T-cell tolerance

- **Active suppression:** Active suppression is thought to occur when non-hematopoietic cells (stimulated by IFN-γ) present antigen in an MHC class II restricted fashion to CD4 T suppressor cells (T_S, also known as CD4+CD25+ regulatory T cells, T_{reg}). Before becoming unresponsive, these cells may induce specific CD8 T_s cells. In turn, these CD8 T_s cells may suppress antigen-specific autoreactive T cells.
- Myeloid derived suppressor cells increase in sepsis. There is a shift away from T_{H1} to T_{H2} characterized by production of IL-10 (produced by T_{reg}) and inhibition of macrophage.

Mechanisms of peripheral T-cell tolerance

- **Ignorance**: Some autoreactive T cells may never encounter self antigen because it may be sequestered from the immune system. Although they may persist in the circulation, they never become activated.
- **Immune deviation:** Under specific circumstances, non-inflammatory T_{H_2} responses could suppress inflammatory (autoreactive) T_{H_1} responses.

T-cell tolerance

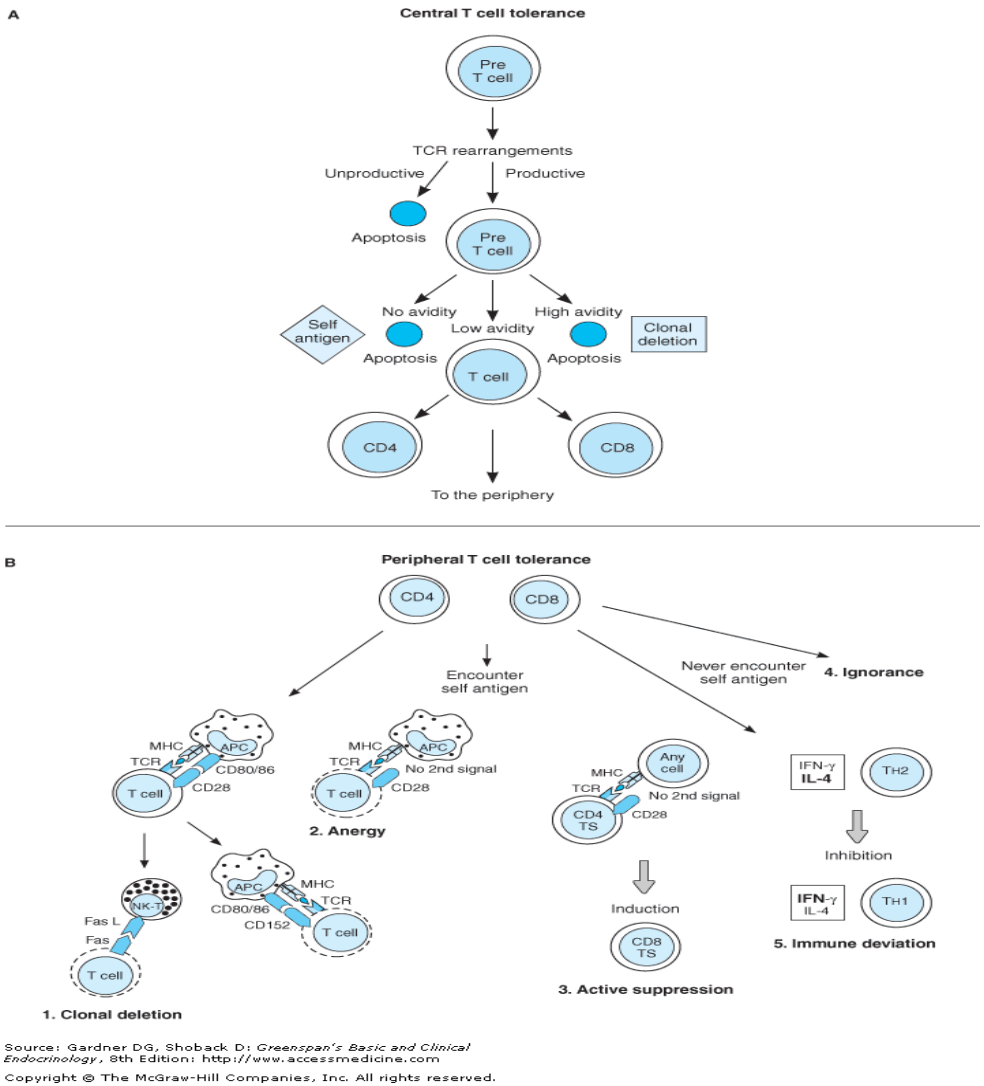

Fig. 3-5 Accessed 07/01/2010

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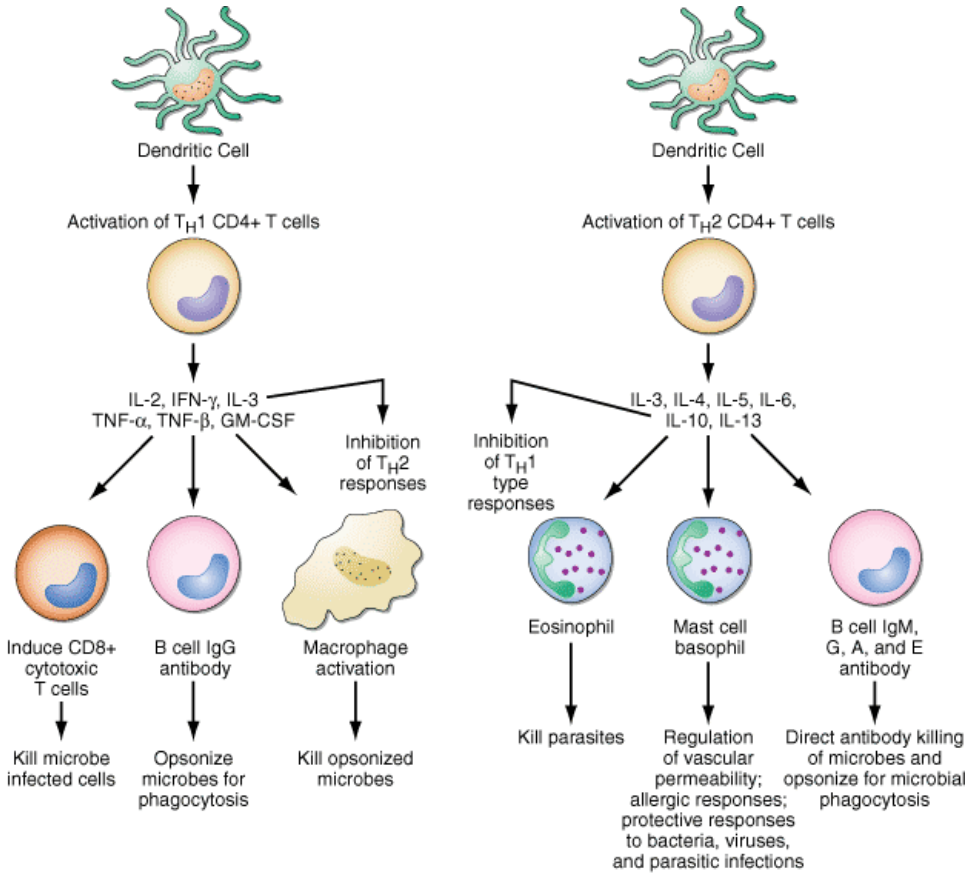
T CELL

- 95% possess TCR αβ.
- CD4+ recognize MHC class II molecules. (CD4)
- T_{H1}, T_{H2} cytokine patterns.
- CD8+ recognized MHC class I molecules (cell processed peptides).
- IL2R-α chain, IL-10, TGF-β characterize suppressor cells. (CD25)
- 5% possess TCR γδ. (C23)
- Recognize low molecular weight polysaccharides from bacterial biosynthesis pathways.
- CD2 is the earliest pan-T-cell marker.

CD4+ cell classes



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Adapted from S Romagnani: CD4 effector cells, in J Gallin, R Snyderman (eds): Inflammation: Basic Principles and Clinical Correlates, 3d ed. Philadelphia, Lippincott Williams & Wilkins, 1999; with permission.)

Fig. 308-2
Accessed 07/01/2010

T_H cell subsets

- T_{H0}
- Secrete IL-3, GM-CSF, IFN-γ, IL-2, IL-4, and IL-5.
- May differentiate into T_{H1} or T_{H2}.
- T_{H1} (Cell Mediated Immunity)
- Delayed type hypersensitivity; development of $CD8^+$ T_C cells.
- Produces IFN-γ, IL-2, TNF-β, IL-3 and GM-CSF.
- Down regulates T_{H2} response via IFN-γ.
- IFN-γ, IL-2 induce

T_H cell subsets

- T_{H2} (Humoral Immunity)
- Help produce antibody. Class switching.
- Promote eosinophil and mast cell production.
- Produce IL-4, IL-5, IL-10, and IL-13.
- Down-regulate T_{H1} responses via IL-10.
- The three subsets are differentiated based on cytokine production as well as cell function.
- IL-4 needed for class switching
- IL-5 induces eosinophil production
- IL-13 induces IgE production

T_H cell subsets

- T_{H17} recruits neutrophils
- Macrophages produce IL-17, IL-22, chemokines
- Production induced by TGF-β, IL-1, IL-6, IL-23

T_H cell activation

- T_H cell and antigen presenting cell exchange mutual activation signals:
- IL-4, IFN-γ from the T_H cell activate B cells and macrophages.
- Macrophages secrete IL-1, IL-6 and TNF-α.
- T_H cell express IL-2R and secrete IL-2.
- Co-stimulatory molecules CD28 (T_H) and B7 (B) as well as adhesion molecules are expressed.
- DNA binding proteins produced regulate the cell cycle.

T_H cell activation

- CD3 is the signaling molecule for T cells (analagous to Igα and Igβ on B cells).
- Composed of 5 proteins lodged in the membrane: γε, δε, and either ζζ homodimer or ζη heterodimer.
- Non-covalently attached to T cell receptor.
- Once the T-cell receptor interacts with MHC ligand, phosphorylation of ITAM on CD3 by receptor associated kinases occurs.

T_H cell activation

- When the co-receptor binds to the MHC ligand, Zap-70 binds to phosporylated ITAM and is phosphorylated and activated
- Increase in intracellular Ca^{2+}.
- T cell proliferates, produces IL-2.
- When no further activation is needed, the APC presents a CTLA-4 protein that interacts more strongly with the CD28 protein, stopping the synthesis of IL-2.

T_C CELL

- CD8+. MHC I restricted.
- Clonal expansion and full effector function requires IL2 (from an activated T_{H1} cell).
- CD80/CD86 co-stimulatory molecules from B-cell. Interact with CD28 and CD 152 (CTLA-4) on T-cell.
- Produces perforins and granzymes that damage target cell.
- Secrete IFN-γ and TGF-β. Trigger metabolic pathway that promotes apoptosis.
- Express FAS ligand and target cells with FAS ligand receptors (promote apoptosis).

Natural killer cells

- Large granular lymphocyte. CD16 (Fc receptor fo IgG confers ability to lyse IgG coated cells) and CD56 receptors on surface.
- No immunologic memory; lack T-cell receptors; lack surface IgM and IgD.
- NKG2D receptors activate.
- Two classes of inhibitory receptors:
- Express C-type lectin (CD94)
- Immunoglobulin like, recognize self-class MHC I. Are not specific.
- Thymus not required for development.

Natural killer cells

- Recognize MHC-α chains (Class I); damage cells that have little MHC I expression. Are not enhanced by exposure.
- Express FAS ligand and target cells with FAS ligand receptors (promote apoptosis).
- IL12 activates, leads to secretion of IFN-γ.
- IL-2 and IL-15 stimulate proliferation of NK cells.
- IgG antibody enhances cell killing.

NK cell receptor activation

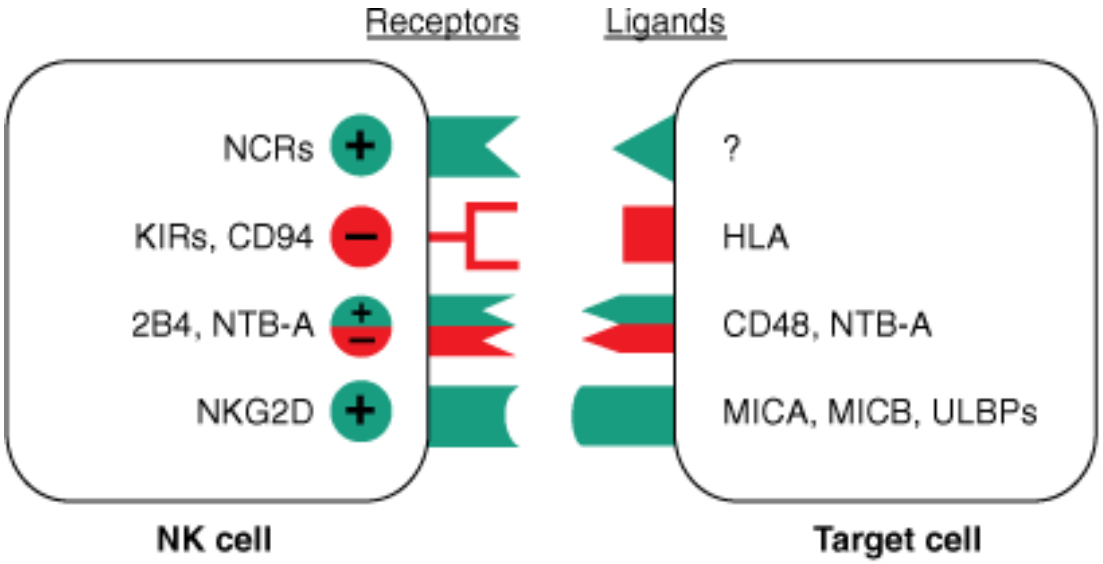

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Fig. 308-4 Accessed 07/01/2010

NK cell killing

- NK cells kill cells that have little MHC class I expression.
- Produces perforins and granzymes (lead to apoptosis).
- FAS ligand on NK cell interacts with FAS on target cell; a Ca^{2+} dependent endonuclease is activated, splitting cellular DNA, inducing apoptosis.
- NK cells have receptors that recognize MHC-α chains and inhibit cell function; receptors that recognize lectins, stimulating cell function.

NKT-T cell axis

- A type I NKT-cell promotes tumor lysis. Type I NKT cells bind to immature dendritic cells (α-galactose ceramide link) via a CD1d receptor and present CD40 ligand as well to those cells. IFN-γ stimulates dendritic cell maturation.
- IL-12 stimulates NK production.
- IL-2 stimulates T_{reg} cell production (feedback on NKT type I production).
- Both CD8 and NK cells (as well as stimulated macrophages) may attack tumor cell.
- The type I cell cross-regulates the type II NK cell.

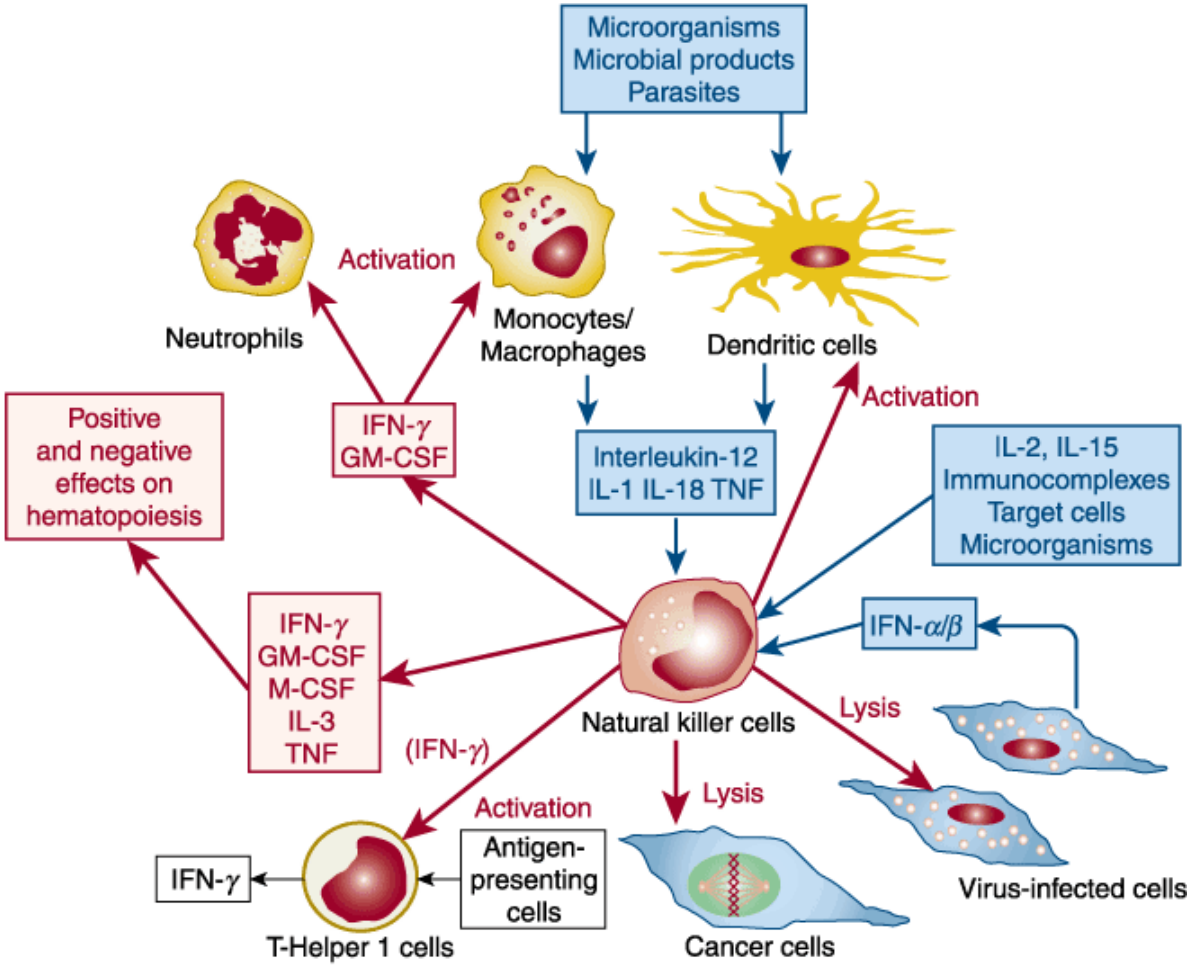
NKT-T cell axis

- A type II cell has CD4 markers. Glycolipid is presented by an antigen presenting cell. IL-13 is produced, activating M2 macrophages as well as CD11+Gr-1+ myeloid cells. Myeloid cells produce TGF-β. All serve to regulate CD8 cell activity and suppress CD8 cell surveillance.

Tumor associated macrophage

- CD163 is a hemoglobin scavenger receptor expressed in monocytes/ macrophages.
- High expression is pro-tumoral and reflects alternate pathway activation.

NK cells



Source: Lichtman MA, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT:
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Fig.79-2 Accessed 07/01/2010

T-cell kinetics

- T cells turnover every 19 hours. Though recruited by IL2, the lag time to division remains unchanged at 2 hours. Recirculation constant between tissues and home sites.
- Unprimed naïve cells: 25CD8+ to 1 CD4+. In the spleen, the ratio is 1:1; in the periphery, 1:2.
- Naïve CD4+ cells have a 118 day half-life in the periphery. If in continuous antigen contact in the periphery, the half-life is 7.5 weeks.
- Naïve CD8+ cells have a 154 day half-life in the periphery. If in continuous antigen contact, in the periphery, the half-life is 41 days.
- Total mature T cells: 3^{11}.

Mechanism of central B-cell tolerance

- As T cells do in the thymus, B cells rearrange their B cell receptor (BCR) in the bone marrow. Unproductive rearrangements drive pre-B cells to apoptosis. Functional rearrangements allow expansion and expression of IgM.
- Next, similar to T cell clonal deletion, immature B cells that strongly bind self antigens in the bone marrow are eliminated by apoptosis. Some autoreactive immature B cells, instead of becoming apoptotic, however, resume rearrangements of their L chain genes, attempting to reassemble new allelic or genes (BCR editing).

Mechanism of central B-cell tolerance

- Soluble self antigens presumably generate weaker signals through the BCR of immature B cells; they do not cause apoptosis but make cells unresponsive to stimuli (anergy). These anergic B cells migrate to the periphery, expressing IgD, and may be activated under special circumstances.
- Only immature B cells with no avidity for antigens become mature B cells, expressing both IgM and IgD. These are the predominant cells that make it to the periphery.

Mechanism of peripheral B-cell tolerance

- In the "absence" of antigen, mature B cells are actively eliminated by activated T cells via Fas-FasL and CD40-CD154 interactions.
- In the "presence" of specific self antigen but "without T cell help," antigen recognition by BCRs induces apoptosis or anergy on mature B cells.
- IL-7, operating through the transcription factor Miz-1, regulates B-cell maturation.

Mechanism of peripheral B-cell tolerance

- If self antigen and specific autoreactive T cell help are provided, two events develop:
- (1) The B cell becomes an IgM-secreting plasma cell, and, in the presence of the appropriate cytokines after expression of CD40 (for T_H cell CD154 interaction), class switching occurs.

Mechanism of peripheral B-cell tolerance

- (2) Further somatic hypermutation of the Ig-variable region genes, which changes affinity of BCRs, occurs. Mutants with low-affinity receptors undergo apoptosis, while improved-affinity BCRs are positively selected. In the presence of CD40 ligation of CD154, antigen-stimulated B cells become memory B cells. These two events are the same as in foreign antigen recognition.

B-cell tolerance

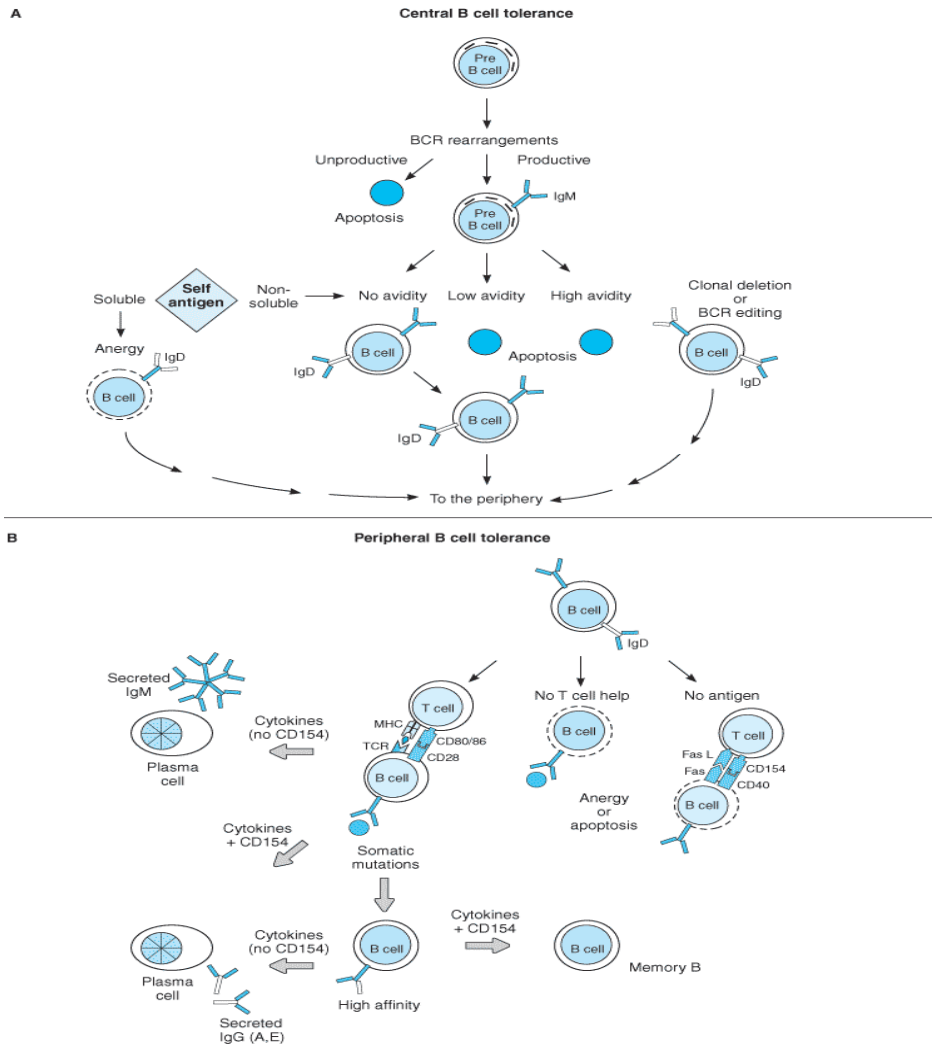

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Fig. 3-6
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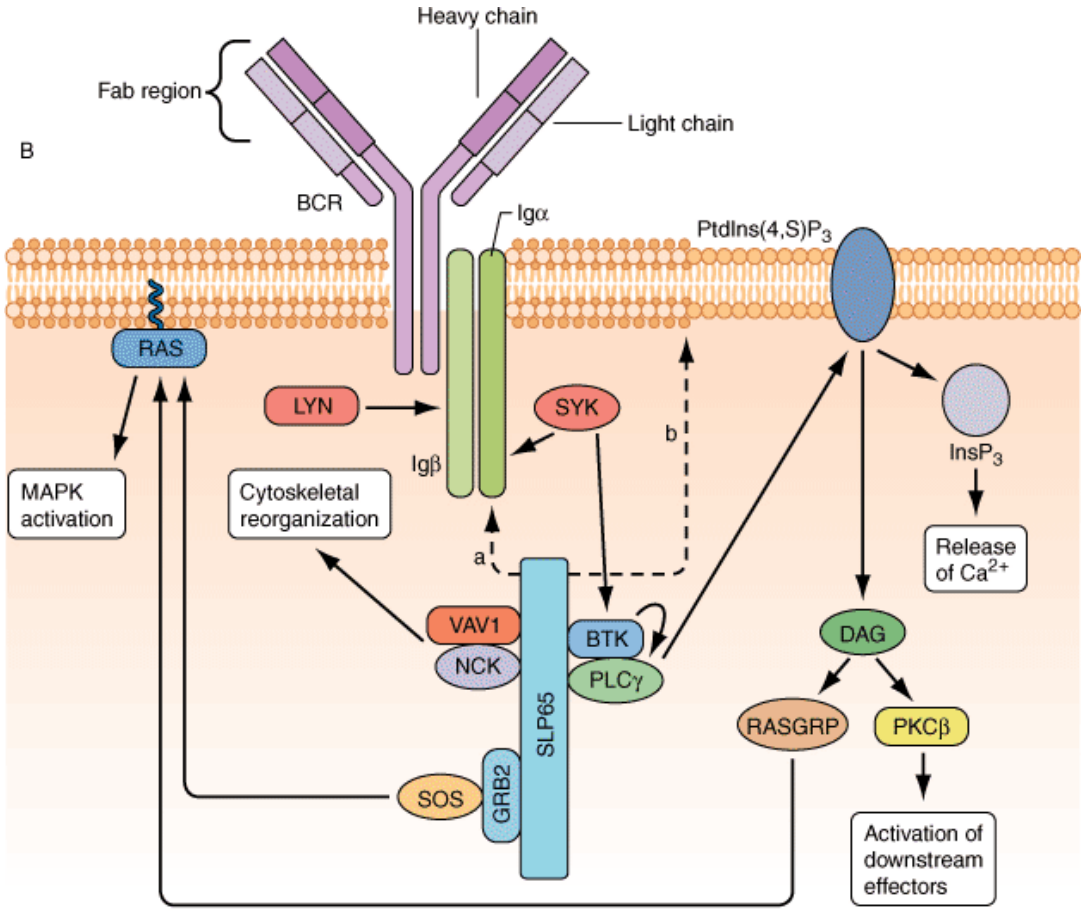
B-cell

- The B-cell receptor for antigen are the heavy and light chain surface immunoglobulins.
- Signal transduction molecules associated with the Ig H chain are Igα (CD79a) and Igβ(CD79b).
- CD21 is the B-cell co-receptor; CD19, and CD81 are additional transduction molecules.
- CD40 interacts with CD154 (CD40 L) on T-cell to induce antibody class switch.
- B7 (CD80 and CD86) are co-stimulatory molecules that interact with CD28 and CD152 (CTLA4) on T-cells.
- CD32 is a low affinity Fc receptor for IgG (Fcγ/RIIb).

B cell receptor activation



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[Adapted from GA Koretzky, F Abtahian, MA Silverman, SLP76 and SLP65: Complex regulation of signalling in lymphocytes and beyond. Nat Rev Immunol 6(1):67–78, 2006; with permission.]

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B cell receptor

- Pre B cell Receptor
- M heavy chains.
- λ5, VpreB.
- Igα and Igβ (signalling molecules).
- B Cell Receptor
- IgM or IgD (naïve B cells only).
- IgG, IgA, and IgE (memory B cells only).
- Immature B cells have only IgM.
- Igα and Igβ (signalling molecules).

Plasma cell and memory cell formation

- Mature B-cell in lymph node contacts antigen presented by T-cell and is activated. Some B-cells establish a germinal center in a lymphoid follicle , “fine tune” the B-cell receptor, and turn into memory cells. They may migrate to the periphery, turn into plasma cells, or remain at site of exposure to await arrival of the same pathogen.

B –cell kinetics

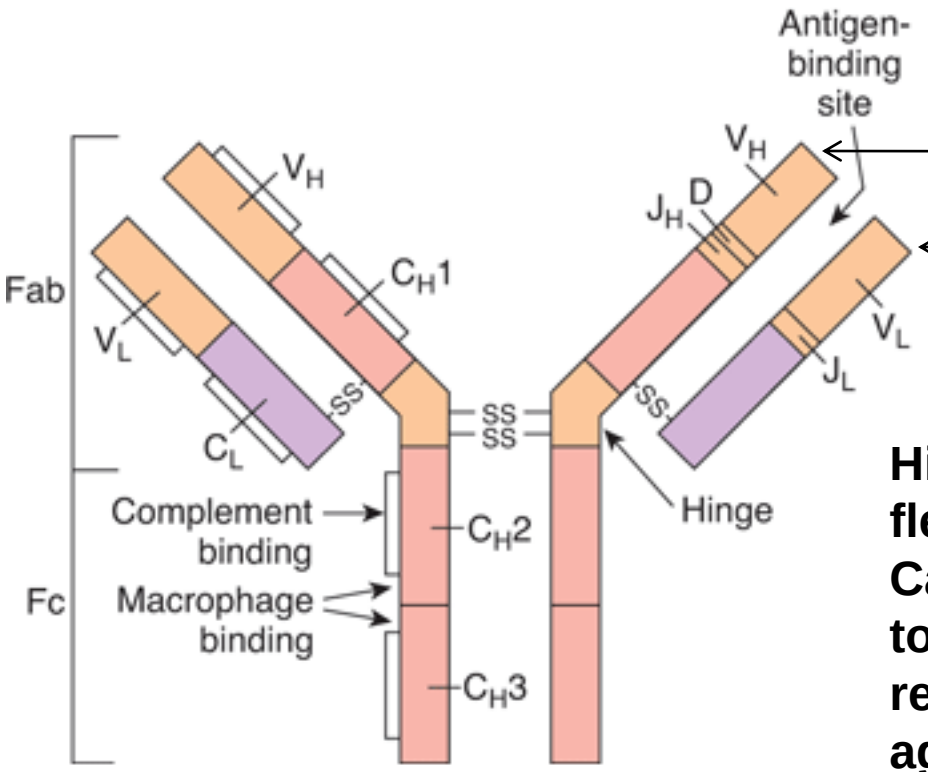
- Naïve cells as well as memory cells have a 5-6 week half-life if not in plasma.
- An unprimed B cell divides monthly; a primed B cell divides every 6 hours.
- A naïve B cell lives 3.5 years.
- A plasma cell lives >1 year even if B-memory cells are present.
- Total mature B cells: 1.3×10^{11}.

Diversity

- Immunoglobulin genes are found in B cells.
- 5 heavy chain classes (μ,δ,γ,α, and ε).
- 2 light chain classes (κ and λ).
- The heavy and light chains are encoded by multiple gene segments.
- Variable (V), Diversity (D) and joining (J) segments compose the variable region of the heavy chain, while only V and J segments are used to generate the light chains.

Immunoglobulin molecule

Fab region binds antibody; Fc region binds complement



Hinge provides flexibility. Carbohydrate bound to complement regions protects against degradation.

Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

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Fig. 3-10 Accessed 07/01/2010

Allelic exclusion

- B cells are diploid.
- A given B cell, however, will only express the rearranged H chain genes from only 1 chromosome and the L chain from only 1 chromosome.
- This ensures antigenic specificity.

Class switching

- This is the hallmark of humoral immunity.
- Occurs after a B cell has come in contact with antigen.
- Requires T cell help (CD40-CD40L).
- After communication with the T_H cell, the B cell rearranges the constant region.
- Irreversible.
- Does not effect variable domain (same specificity maintained).

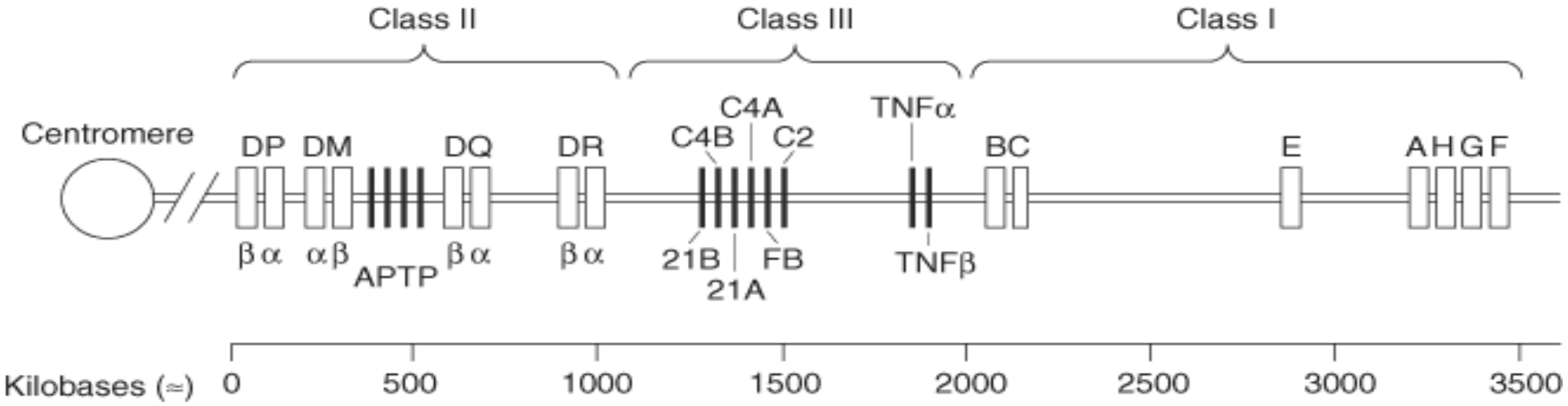
Affinity maturation

- In the course of a humoral response, the average affinity of the antibodies produced increases by 100-10,000 fold as a result of point mutations, deletions, or insertions into the V,D, or J region of rearranged immunoglobulin genes, and the selection of high affinity clones.
- Higher affinity antibodies are positively selected; lower affinity antibodies are signaled to die by apoptosis.

Isotype	Serum Form	Subclasses	Functions
IgG	monomer	IgG1 IgG2,3,4	Produced in secondary response (high affinity); activate classic complement pathway; crosses placenta via Fcγ receptors; opsonizes antigen for uptake by Fcγ receptors; on memory B cell as antigen receptor
IgA	monomer	IgA1 IgA2 is dimer with J chain in secretions	Primary antibody in mucosal secretions; J chain prevents degradation of dimer on mucosal surface; on memory B cell as antigen receptor
IgM	pentamer		Monomer on memory B cell as antigen receptor; secreted as pentamer during primary response (high avidity); activates classic complement pathway; first antibody produced in neonates; on naïve B cell as antigen receptor
IgD	monomer		Membrane bound on mature B cells; on naïve B cell as antigen receptor; never secreted
IgE	monomer		Bound to surface of mast cells and basophils by Fcε receptors (triggers degranulation); on memory B cell as antigen receptor

HLA complex

- **Regions encoding the three classes of MHC proteins on top. APTP denotes a cluster of genes within the class II region encoding genes related to antigen processing, transport, and presentation. Class III region encodes genes unrelated to class I or class II not involved in antigen presentation (TNF-α and -β, complement factors C2, C4, B, and 21-hydroxylase and others).**



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HLA class I (A,B,C)

- MHC I molecules are found on all nucleated cells apart from sperm, neurons. Each allele of the MHC complex encodes for a single α-chain. The α-chain confers antigen specificity. There is a hydrophobic trans-membrane domain.
- MHC I molecule is a heterodimer consisting of a polyvalent heavy chain (α) with three extracellular domains. The α_1 and α_2 domains form a peptide bounding cleft that faces outward.
- The α_3 domain has binding site for CD8.

HLA class I (A,B,C)

- The α-chain is noncovalently bound to β2-microglobulin (coded outside the major histocompatibility complex). β-2 microglobulin is required for proper folding of the molecule.
- Responsible for presentation of endogenous antigens (viral). Protein degradation of the antigen intracellularly generates a peptide of 8-10 amino acid length that is bound in the cleft for presentation to T_C cells.

HLA class II (D)

- Expressed on antigen presenting cells.
- MHC II molecule is a non-covalently bound heterodimer. Composed of α and β chains each of two domains. (both encoded in the major histocompatibility complex).
- Highly polymorphic glycoproteins.
- Hydrophobic transmembrane region.

HLA class II (D)

- Juxtaposition of the α1 and β1 domains not covalently bound at the cell surface form an outwardly facing cleft that binds peptides of 12-15 amino acids in length. Invariant chain prevents endogenous peptides from binding in the peptide binding groove.
- Responsible for presentation of exogenous antigens (extracellular bacteria) to T_H cells. β_2 domain has binding site for CD4.

Cytokine and viral regulation of MHC expression

- Interferon-γ and tumor necrosis factor can increase the expression of class II MHC by inducing the formation of specific transcription factors that bind to promoters of MHC genes.
- MHC expression is also increased by some viruses (Cytomegalovirus, Hepatitis B virus, and Adenovirus). More commonly MHC expression is decreased.
- CMV proteins can bind to β2 microglobulin, preventing the proper assembly of class I MHC molecules.

Acquired immunity

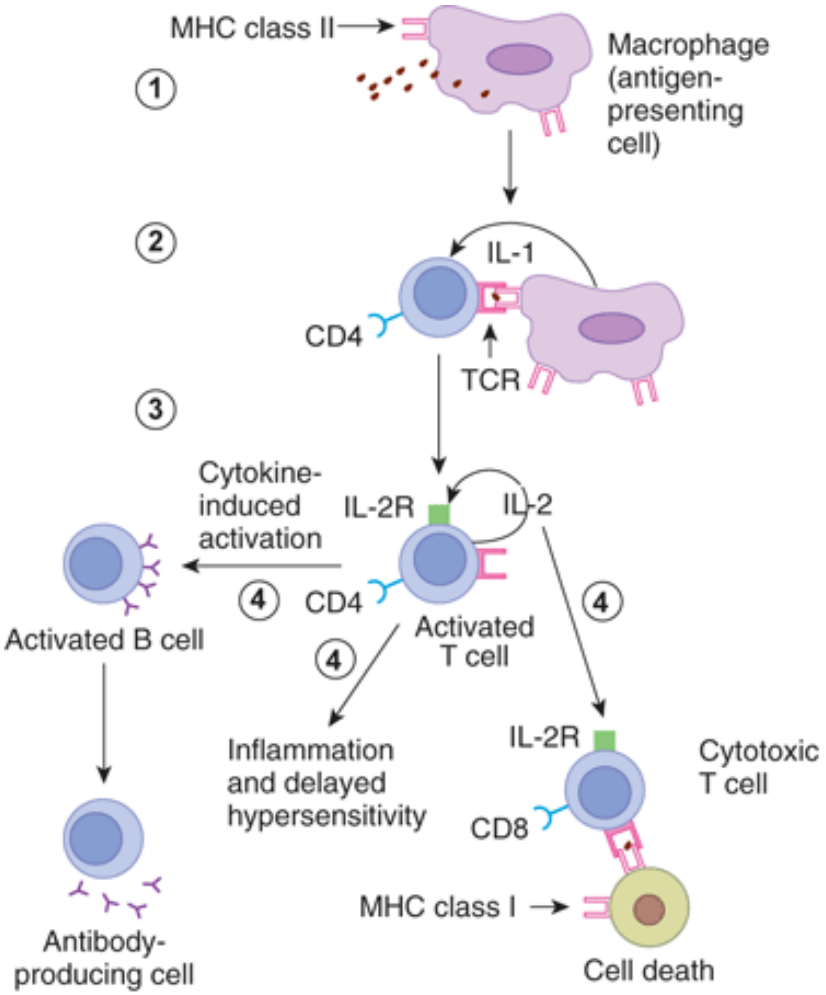

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Fig. 3-9 Accessed 07/01/2010

Antigen presenting cells

- Macrophages, B cells, Langerhans, and interdigitating dendritic cells are the only cells that can present antigens to T_H cells.
- Antigen presenting cells express a molecule (B7) that is only constitutively expressed on interdigitating dendritic cells.
- Interdigitating dendritic cells are the only cells that can activate naïve T cells.
- Macrophages can only activate naïve T cells when activated by IFN-γ.
- B cells can activate naïve T cells after contact with antigen.

Antigen modulation

- PD-L1 expressed on both dendritic cells and macrophages. Binds to CD28 and interacts with homologue of B7 and stimulates T_{reg}.
- CTLA4 (CD152) also present on dendritic cells. Binds to CD86 and interacts with homologue of B7 and inhibits T_{reg}.
- B and T lymphocyte attenuator (BTLA, CD272) is induced by T cell activation. Expressed on T_{H1}. As with PD-L1 and CTLA4, interacts with B7 homologue but inhibits T cell (CD8+) via TNFR.

Antigen processing

- Macrophage engulfs antigen (endocytosis). In lysozome. The release of cytokines IFN-γ, from T_{H1} (T_C cells) activates macrophages.
- Antigen cleaved to peptides.
- MHC class II molecule combines with peptide and migrates to cell surface.
- Helper T cell (CD4) T cell receptor recognizes macrophage with antigen because of presence of MHC II molecule with antigen on cell surface.
- Helper T cell secretes IL-2 which recruits B cells.
- Plasma cells secrete IgG, opsonize bacteria.

Antigen processing

- Helper T cells also secrete TNF and IFN to activate cytotoxic T cells (CD8).
- Cytotoxic T cells attack intracellular antigen when MHC type I molecule presents antigen on cell surface.
- Cytotoxic T cell does not recognize intracellular antigen without MHC I molecule.
- Cytotoxic T cell recognizes antigen attached to MHC I inside cell because of presence of MHC I and antigen on cell surface.

Multispecific recognition

- Multidomain proteins participate in permanent interactions such as macromolecule assembly. Slowly evolve. Multiple-interface signaling hubs.
- Multispecific recognition proteins manifest in protein-protein, protein-DNA, antibody-antigen, and TCR-peptide interactions. Single-interface signaling hubs.
- p53, p21, p27, calmodulin, ubiquitin, thioredoxin, RAS as examples of protein network single-interface signaling hubs. Evolve at same rate as whole proteome.

Multispecific recognition

- Intrinsically disordered. Disordered regions frequently fold upon target recognition sites and may be flexible linkers that permit other binding domains to assume different orientations with respect to each other.
- Conformational flexibility characteristic (orientation of side chains, loop flexibility, alteration of local secondary structure, as well as conformational change). However, small molecule binding does not require conformational change.
- High surface charge present. Electrostatic forces may optimize orientation of the receptor protein for particular partners.

T-cells and MHC receptors

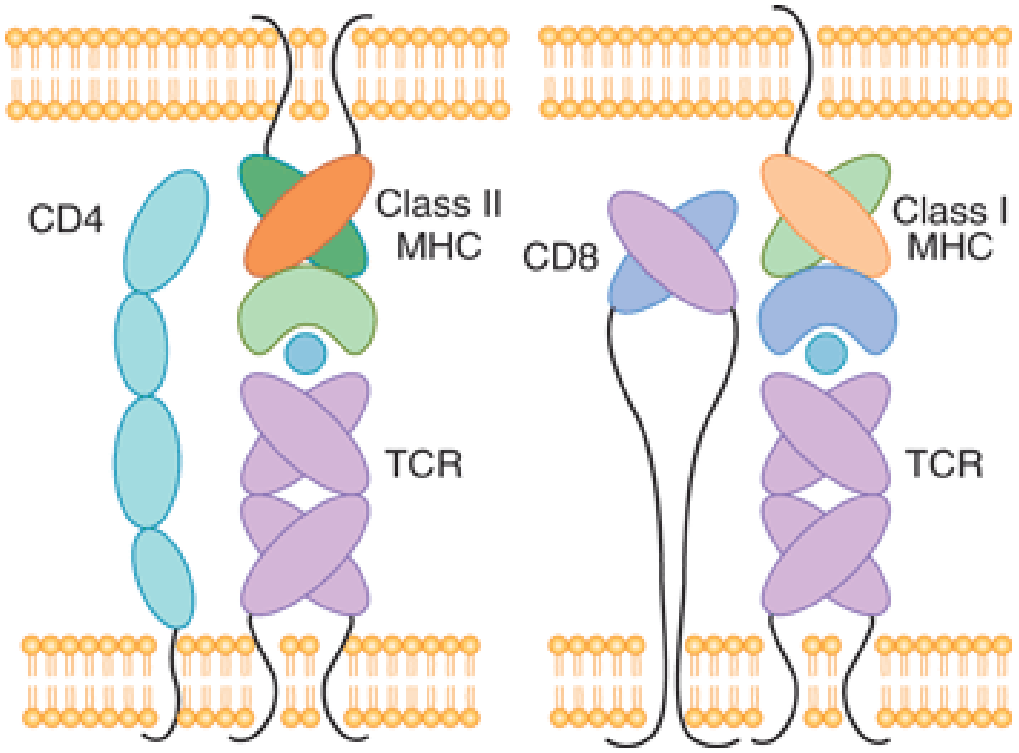

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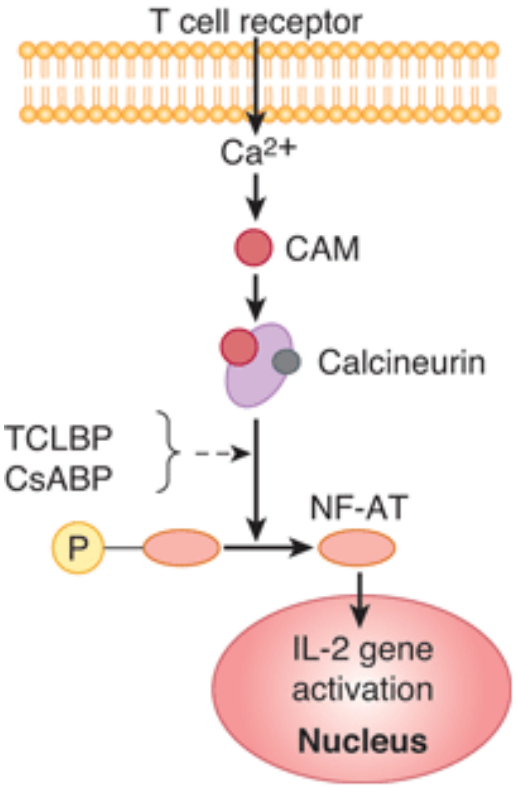
Fig. 3-8 Accessed 07/01/2010

Acquired immunity

- An antigen-presenting cell ingests and partially digests an antigen, then presents part of the antigen along with MHC II peptides on the cell surface.
- An "immune synapse" forms with a naive CD4 T cell, which is activated to produce IL-2.
- IL-2 acts in an autocrine fashion to cause the cell to multiply, forming a clone.
- The activated CD4 cell may promote B cell activation and production of plasma cells or it may activate a cytotoxic CD8 cell. The CD8 cell can also be activated by forming a synapse with an MCH I antigen-presenting cell.

IL-2 activation



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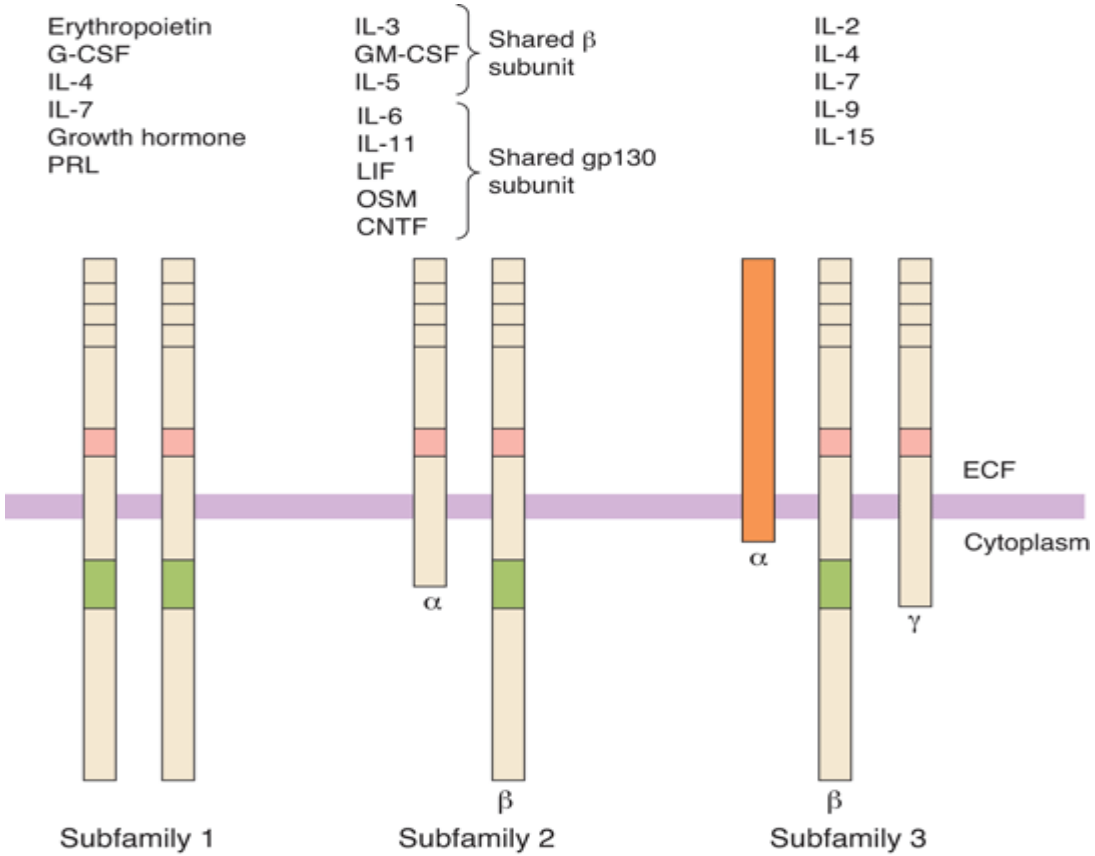
Cytokine receptor superfamilies



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(Modified from D'Andrea AD: Cytokine receptors in congenital hematopoietic disease. *N Engl J Med* 1994;330:839.)

Fig. 3-4 Accessed 07/01/2010

Important cytokines

Cytokine	Major Source	Major Function
IL-1	**Macrophages**	**Proinflammatory cytokine; activates CD4 and endothelial cells**
IL-2	**T_{H1} subset of T cells**	**Activates B, CD4 and CD8 cells.**
IL-4	**T_{H2} subset of T cells**	**Stimulates B-cell growth Increases T_{H2} subset of CD4 cells Increases isotype switching and IgE**
IL-5	**T_{H2} subset of T cells**	**Stimulates B-cell differentiation Increases eosinophils and IgA**
IFN-γ	**T_{H1}subset of T cells**	**Increases class I and II MHC expression Stimulate phagocytosis and killing by macrophages and NK cells**
TNF-α	**Macrophages**	**Proinflammatory cytokine Activates neutrophils and endothelial cell adhesion**

Important cytokines

Type	Major Source	Function
IL-6	**CD4 cells and macrophages**	**Stimulates B-cell differentiation Acute phase reactant**
IL-8	**Activated mononuclear cells**	**Attracts neutrophils (Cysteine-amino acid- cysteine characterizes polypeptide; α-class chemokine)**
IL-10	**Th-2 subset**	**Regulate T_{H1} production by limiting IFN-γ production**
Il-12	**Th-1 subset**	**Promotes development of T_{H1}subset**
TGF-β	**CD4 T cells**	**IgA switch; stimulate B cells, macrophages, neutrophils; diminish T cell function**

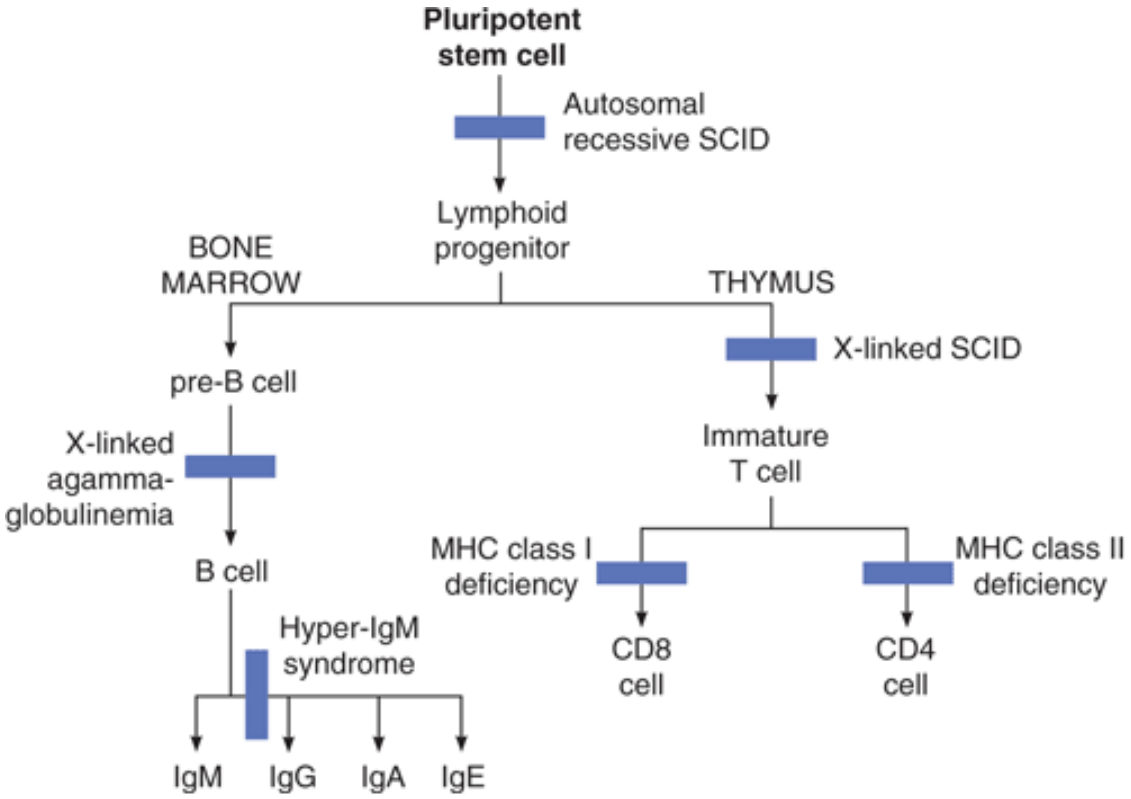
Other important cytokines

Type	Major Source	Function	
IL-13	Th-2 subset	Binds to receptor that shares chain with IL-4 receptor	Airway hyperresponsive
RANTES MCAF	Activated T cells	Attracts monocytes	Cysteine-- cysteine characterizes polypeptide β-class chemokine
IL-3	Activated CD4 cells	Stimulates stem cell growth and differentiation	
GM-CSF	T lymphocytes and macrophages	Stimulates neutrophil and macrophage growth	
IFN-α IFN-β	Leukocytes Fibroblasts	Block viral protein synthesis (inactivating elongation factor; activate RNA endonuclease)	May be induced by double stranded RNA as well

Hypersensitivity reactions

Type	Mediator	Reaction
Anaphylaxis I	**IgE**	**Degranulation of mast cells and basophils by allergen sensitized bound IgE. Histamine, leukotrienes, tryptase released. Eosinophil chemotaxis occurs.**
Antibody mediated cytotoxicity II	**IgG**	**Complement mediated lysis of antigen-antibody coated cells in circulation or in extracellular matrix.**
Immune Complex III	**IgG**	**Antigen-antibody complexes deposited in basement membranes and capillary venules, activating complement. Neutrophils attracted to site. Lysozymal injury.**
Delayed IV	**T Cell**	**Antigen sensitized CD4 cells release lymphokines, inducing inflammation and activating macrophages. (May see CD8 mediation in contact dermatitis.)**

Immunodeficiencies



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(Modified from Rosen FS, Cooper MD, Wedgwood RJP: The primary immunodeficiencies. *N Engl J Med* 1995;333:431.)

Fig. 3-12
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