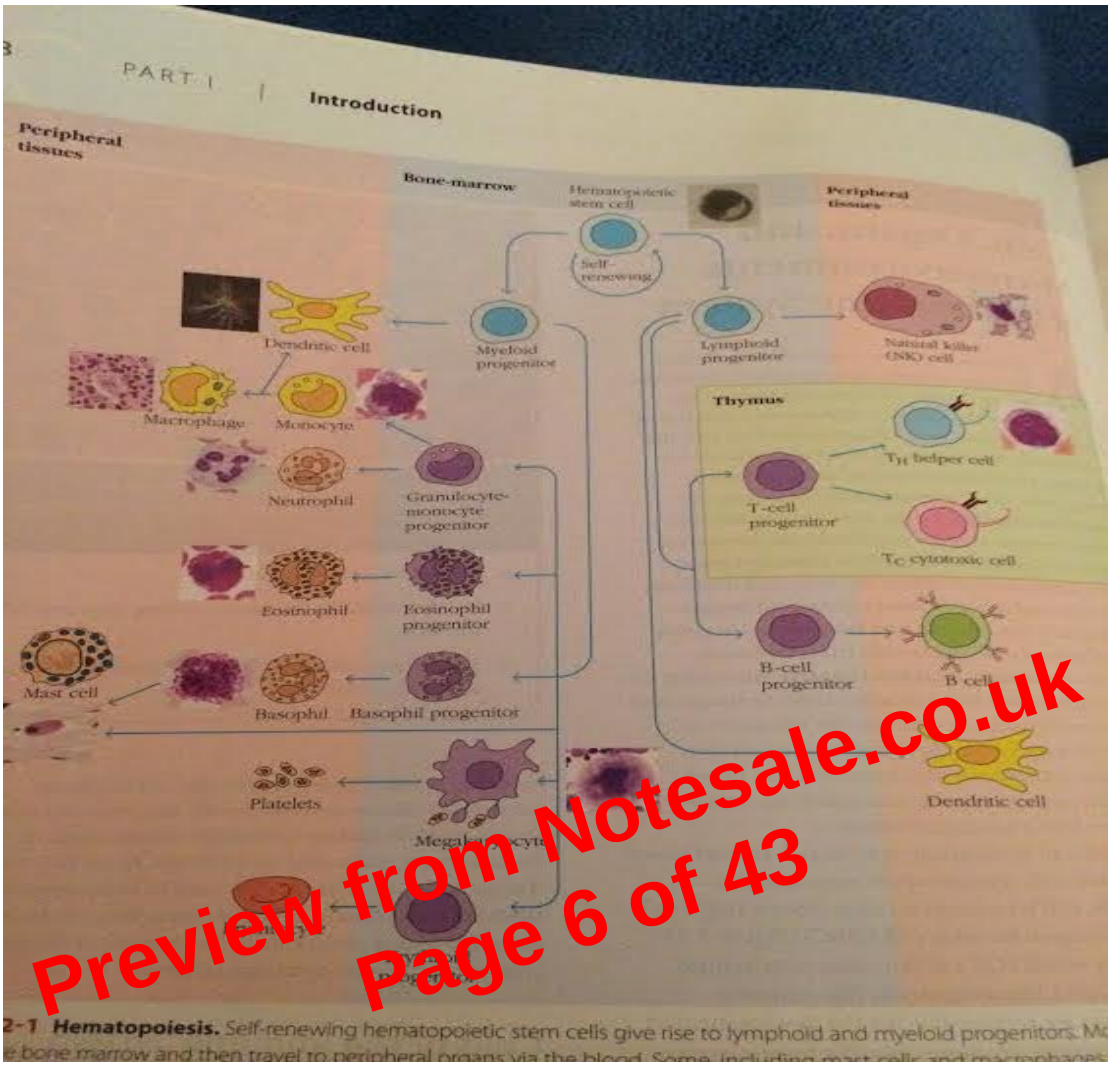

- Adult hematopoietic stem cells give rise to all blood cells (leukocytes, RBCs, and platelets)
    - All arise in bone marrow
    - One of adult stem cells always becomes another stem cell and then either becomes myeloid progenitor or lymphoid progenitor
        - Basophils- blue stain with hemotoxulin
        - Eosinophils- red stain with eosin
        - Neutrophils don't stain
- In a sample of 100 leukocytes, there will be 60-70 neutrophils (multilobed nucleus), 30-40 lymphocytes (nucleus almost fills whole cell), 3 to 5 monocytes (large), 1 to 3 eosinophils (purple nucleus, red granules), and less than 1 basophil (purple/blue granules).
- Basophils and mast cells have histamine (released in allergies/asthma)
    - Degranulation- opens capillary beds and contraction of smooth muscle in trachea
- Chemokines (chemical that attract other cells) and cytokines are released to activate other cells (macrophages to present antigens)

    - Hydrogen bonds, electrostatic interactions, hydrophobic interactions, Van der Waals interactions
    - All relatively weak, especially in H_2O, but many weak make it strong enough to stay long enough to initiate signal transduction
- Monovalent ligand-only one ligand and receptor
- Multivalent ligand- multiple connected ligands (majority)
    - While one is attached, other one stays in vicinity
- Heterodimeric- Two polypeptide chains different from each other
- Packets of IL-12 by dendritic cells secreted in direction of a bound T cell
- 3 Pathways to alter gene expression
    - A ligand binds a receptor with receptor-associated molecules and coreceptors (have receptor-associated molecules because receptor has large extracellular domain but very small intracellular domain). A lipid raft is a place in the plasma membrane where molecules (mostly proteins) aggregate with specialized function. Lyn in the lipid raft is a Src protein (tyrosine kinase) that phosphorylates ITAMs, that serve as a docking center for adapter proteins, like BLNK, that brings other proteins such as Syk that lead to signal transduction. This recruits phospholipase C, which cleaves PIP_2 into DAG and IP_3 and IP_3 releases Ca^{2+} from the ER and Ca^{2+} bind calmodulin, which activates calcineurin. This dephosphorylates NFAT (nuclear factor of activated T-cells). NFAT can then move through the nuclear pore and can act as a transcription factor.
        - CD19/21 are also phosphorylated by Lyn, which activates PI_3K, which allows PDK2 to associate with Akt. This phosphorylates Bax and Bad, inactivating them from pro-apoptotic to anti-apoptotic (cell survival and proliferation)
        - Cyclosporine with cyclophilin inhibit the calcitonin/calcineurin pathway, preventing dephosphorylation of NFAT. This does not allow for proper maturation and differentiation of T cells, ultimately inhibiting tissue rejection.
    - DAG remaining in the membrane after PLC cleavage of PIP_2, binds and activates PKC, which phosphorylates and activates enzymes that destroy IkB, the inhibitor of NF-kB. This allows NF-kB to enter the nucleus and act as a transcription factor.
    - Binding of Ras-GRP to DAG in the plasma membrane activates the GEF SOS (Son of Sevenless) to activate Ras. Ras then activates the MAP kinase cascade, which ends in AP-1, a transcription factor that enters the nucleus.
- Tyrosine kinase domain is inactive and separate until a growth factor binds that brings them closer and kinase activity is stimulated by cross-phosphorylation
    - Tyrosine is first phosphorylated, and threonine and serine later
- Lipid raft- a place in the plasma membrane where proteins (molecules) aggregate with a specialized function

    - Receptor not in lipid raft unless bound to ligand
- Coreceptors for T-cells are CD 4/8 and receptor-associated molecules are hexameric C3d (delta, epsilon, zeta, zeta, gamma, epsilon)
- Coreceptors for B-cells are CD 19/21 and receptor-associated molecules are Ig alpha and beta
- MHC's are known as promiscuous because they can bind up to 500 different antigens
- ITAMs (Immunoreceptor tyrosine-based activation motif) help increased intensity of signaling and is where signaling takes place
- SH2 (Src-homology 2) domain on protein is usually inactive due to inhibitory phosphate group on a tyrosine of SH2. However, the presence of Csk (C-terminal Src kinase) dephosphorylates this tyrosine, which causes it to become active, further stimulating Src kinase activity.
    - SH3 binds proline preferentially
- PIP_3 (phosphotidylinositol trisphosphate)
    - Phospholipase-gamma-1 (B-cells) and –gamma-2 (T-cells)
- Calmodulin can bind 4 Ca^{2+}
    - Activates and opens up, which then allows it to bind calcineurin which dephosphorylates NFAT
- Experiment where electrophoresed fluid (plasma) of blood and found 4 major peaks (highest peak was albumin, which is major protein in all blood)
    - Major peaks were albumin as well as IgG
    - IgG most common Ig in blood, because IgA found in mucosa
- An immunoglobulin contains 2 heavy and 2 light chains
    - The tail is the Fc (constant region- genetic consistency)
- 3 Ways to Digest an Immunoglobulin
    - Papain digestion- cleavage in hinge area, but does not destroy disulfide linkages (separates Fab's and Fc)
    - Pepsin digestion- Get $F(ab')_2$ because keeps all 4 disulfide linkages with 2 binding sites on one fragment
    - Mercaptoethanol reduction- breaks all parts (S=S become S-H)
- Immunoglobulins typically have carbohydrates bound to them
    - More soluble in H_2O (important because in blood or interstitial fluid)
    - Help separate Fc regions (important to interact with complement)
        - Fc named because binds complement and crystallizes when cut with papain
- Chain composition of the 5 immunoglobulin classes
    - IgG has a gamma heavy chain, 3 C_H Ig domains, gamma (1,2,3,4) subclasses and no J chain
    - IgM has a mu heavy chain, 4 C_H Ig domains, no subclasses and a J chain
    - IgA has an alpha heavy chain, 3 C_H Ig domains, alpha (1,2) subclasses and a J chain
    - IgE has an epsilon heavy chain, 4 C_H Ig domains, no subclasses and no J chain

- CLR (C-type lectin receptor), RLR, and NLR signaling pathways
    - CLRs are plasma membrane receptors on monocytes, macrophages, dendritic cells, neutrophils, B and T cells, that bind carbohydrate components of fungi, viruses, and allergens
    - RLRs are soluble PRRs that reside in the cytosol and sense viruses/intracellular parasites (in case miss and they enter cell through endosome)
    - NLRs (nod-like receptors) are cytosolic and are activated by PAMPs/DAMPs
        - Usually activates signal transduction through 1 of 4 pattern recognitions (LPS, flagellin, etc.)
    - CLRs, RLRs, and NLRs function to activate their cells to produce antimicrobials, cytokines, chemokines, and IFN-å/ß
    - CLR signaling pathway
        - Ligand binding to a CLR causes its ITAM to be phosphorylated, which recruits Syk. Syk triggers the MAP kinase cascade, producing AP-1. Syk also activates CARD9, which activates the IKK complex, leading to NF-kB activation.
- Some NLRs do not trigger signaling pathways. Instead, they assemble with other proteins into a complex, the inflammasome, which activates proteases. These proteases activate pro-inflammatory IL-1ß and IL-18.
    - These are inactive until cleavage because we don't want them inside our cells (they are typically secreted)
- Inflammasomes
    - Can be activated by DAMPs such as ß-amyloid (associated with Alzheimer's plaques)
    - Associated with serious inflammatory conditions caused by urate crystals, which cause gout (complex form of arthritis)
    - Acute inflammation is ok, but not chronic
- Cytokine IL-1 acts on the hypothalamus to cause fever (inflammation)
- Chemokine IL-8 chemoattracts cells to infection site
- Major antiviral activities induced by Type 1 IFNs
    - INF-å/ß binds IFN receptors and dimerized STAT causes transcription of Protein kinase R (PKR), which destroys dsRNA and inhibits translation by blocking elongation factors. Mx factors polymerize in the cytosol and inhibit viral transcription and assembly.
- Initiation of a local inflammatory response
    - Tissue damage and bacteria cause sentinel cells to release chemoattractants that trigger an increase in local blood flow and capillary permeability. Permeable capillaries allow an influx of fluid and cells. Neutrophils and other phagocytes migrate to site of inflammation (chemotaxis) and destroy the bacteria.

    - Every 200 meiotic divisions, crossing over of MHCs occur
- Class II are polygenic and polymorphic
    - Polygenic- more than one gene codes for similar products (DP, DQ, and DR code for similar α and β domains)
    - Polymorphic- There are multiple alleles of each gene
- HLA-G from class I MHCs protects the fetus (paternal antigens recognized by maternal T cells)
- Syngeneic- identical at all genetic loci (only MHC loci identical)
- Congenic- genetically identical except at one allele (can be used in experiments to see how immune system works)
- Skin transplantations only work if the recipient has the MHC haplotype that the donor does. Otherwise, the skin graft will be attacked as foreign
    - A new haplotype can arise from rare recombination of a parental haplotype
- MHCs are promiscuous- can bind up to 500 different segments
    - There are 10^{25} different MHC molecules, but a typical human only expresses about 12 because born with a haplotype
    - This is why humans can respond to almost any antigen, even if it has never been seen before (so much diversity in MHCs, BCRs, and TCRs)
- Both maternal and paternal MHC genes are expressed (codominant expression)
    - Because class II molecules are heterodimers, new molecules with one maternal and one paternal chain are produced (increases diversity)
- Bottleneck is a problem with the cheetah population because they have such few numbers that their MHC molecules are nearly identical. Therefore, if a certain pathogen is able to kill one of them, it could kill all of them
- In class I HLAs, most of the variable residues are in the α_1 and α_2
    - Increased variability and mutations in the peptide-binding region
- Ankylosing spondylitis- polymorphisms in peptide-binding region don't allow for proper protein binding (90% HLA-B27+)
- Cytotoxic T cells kill tumor, aging, or cells carrying intracellular parasites
- Professional APCs
    - Dendritic cells, macrophages, B cells
        - Always presenting
        - Dendritic cells don't even have to be activated
            - Always express costimulator on membrane/high levels of MHC
        - Macrophages only have high levels of MHCs when activated
        - B cells need stimulation but high MHC levels
- Nonprofessional APCs
    - Fibroblasts, glial cells, pancreatic beta cells
        - Not always presenting
- All nucleated cells have MHC I, because looking for viruses (intra. parasites)

- Roles of MHCs
    - Clear intracellular parasites, make antibodies (mop up virus that escapes from cell), activate cytotoxic T lymphocytes, select to eliminate auto-reactive T cells, tolerance, recognize tumor and virus-infected cells (because they downregulate MHC II)
- There is no somatic mutation in mature T cell, so essential to get rid of auto-reactive T cells in thymus
- Self-MHC restriction of T_H cells
    - Peritoneal exudate cells were taken from a strain 2, 13, and progeny of a 2x13 cross guinea pig in order to obtain peritoneal macrophages. Antigen was then added to create "antigen-pulsed macrophages". A strain 2, 13, and progeny of a 2x13 cross guinea pig were exposed to the same antigen and, after 7 days, lymph node cells were extracted in order to obtain antigen-primed T cells. T cell proliferation was then assessed. T cell proliferation only occurred when antigen was presented by macrophages that shared MHC alleles.
    - A classic experiment shows that T cells don't recognize a different MHC allele (same thing as above: self-MHC restriction of T_H cells)
- Endogenous pathway- How most intracellular parasites and self get presented (cytotoxic T lymphocytes)
- Exogenous pathway- Bacteria and escaped viruses (antibodies)
- Experiment showing antigen processing is necessary for T_H activation
    - If the APC is fixed and then incubated in antigen for an hour, no T cell activation occurs
    - If the APC is incubated in antigen for an hour, and then fixed, T cell activation occurs
    - If the APC is fixed and then incubated with antigen peptides for an hour, T cell activation occurs
- Chloroquine- Known for killing the *Plasmodium* that caused malaria by raising pH of lysosomal compartment (now resistant to it)
- Leupeptin- protease inhibitor
- Immunoproteasome must be able to cleave peptide so will bind MHC I
    - Helps facilitate right size and amino acid constituency to bind β-floor and α-helices
    - Targeting by proteasome requires Ubiquitination
- ATP-binding casette transporters have a large extra-membrane domain with multiple transmembrane passes (TAP1/TAP2 heterodimer)
    - Proteins are degraded by the immunoproteasome and the resulting peptides leave through TAP1/TAP2 heterodimer into the RER lumen where they will associate with class I MHCs
- Assembly and stabilization of MHC I molecules
    - An MHC I α-chain associates with calnexin and ERp57 on the inside of the RER membrane until β-microglobulin binds the α-chain. This binding causes the release of calnexin and allows binding to calreticulin and tapasin, which are associated with the peptide-

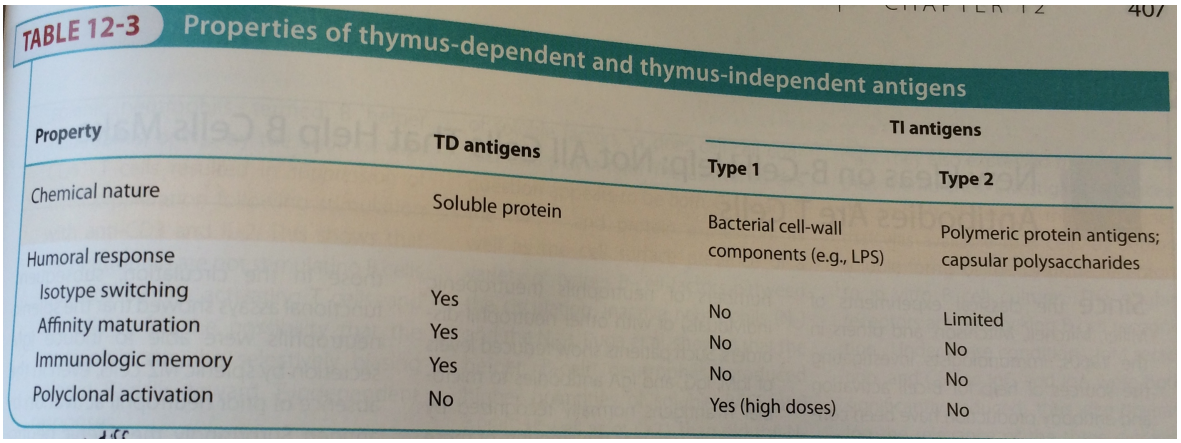

TABLE 12-3 Properties of thymus-dependent and thymus-independent antigens

Property	TD antigens	TI antigens	
		Type 1	Type 2
Chemical nature	Soluble protein	Bacterial cell-wall components (e.g., LPS)	Polymeric protein antigens; capsular polysaccharides
Humoral response			
Isotype switching	Yes	No	Limited
Affinity maturation	Yes	No	No
Immunologic memory	Yes	No	No
Polyclonal activation	No	Yes (high doses)	No

(Ch. 13)

- Four broad categories of antibody effector functions
    - Antibodies can neutralize pathogens by binding to and blocking receptors that pathogen uses to gain entry into a cell
    - The Fc region of antibodies bound to antigen bind the FcR of phagocytic cells, inducing internalization and degradation (opsonization)
    - Antibodies can recruit and activate complement proteins that can either directly kill the pathogen or form the MAC
    - Antibodies can bind to FcRs on cytotoxic cells (NK cells) and direct their activity to infected cell, tumor cells, and pathogens
- IgG1/2/3, E and IgD have 150,000 kD. IgA1/2 have 150,000-600,000 kD. IgE has 190,000 and IgM has 900,000. Only the IgGs cross the placenta
- IgG and IgM activate classical complement pathway; IgM better because more binding sites; B1 and B2 B cells are both plasma cells but B1 makes the majority of IgM
- Herceptin is a monoclonal antibody used in breast cancer; works by binding growth factor so can't bind receptor
- FcR Functions
    - When bound by IgE-pathogen (worms) complexes, the FcR can induce release of histamine (dilates smooth muscle and constricts airways) and proteases (degranulation)
    - When bound by antibody-pathogen complexes (IgG or IgA), can induce macrophage activation and phagocytosis (opsonization)
    - The neonatal FcR (FcRn) binds antibody that has been nonspecifically engulfed by endothelial cells and return it to the blood intact
    - When bound by IgG antibodies coating infected or tumor cells, FcRs activate NK cells
    - PolyIg receptors (PolyIgR) expressed by the basolateral surface of epithelial cells will bind dimers and multimers of IgA and IgM and transfer them into the lumen of an organ (transcytosis)
        - Accumulation of antibodies in bodily secretions