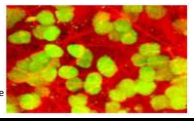
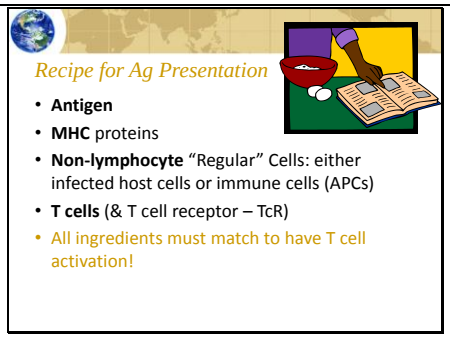
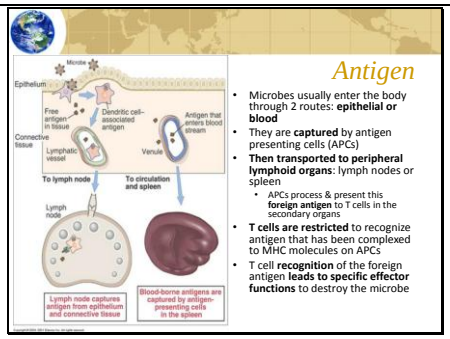
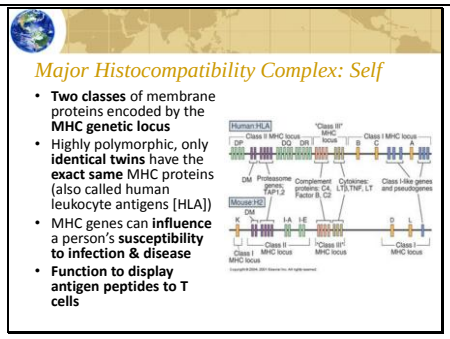
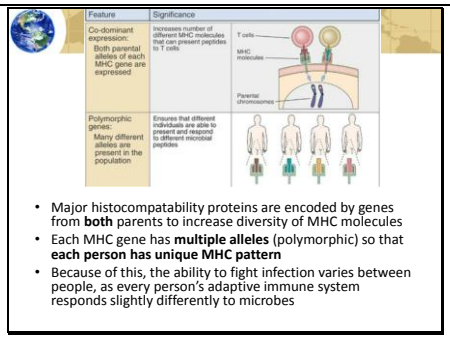
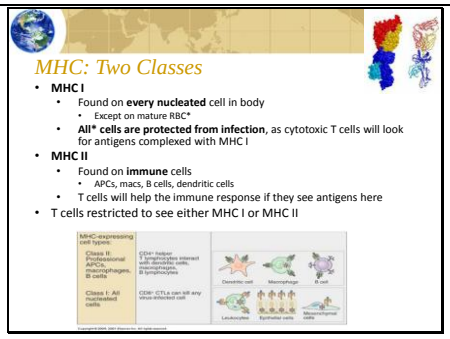
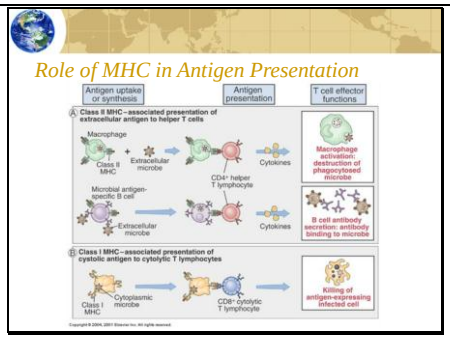
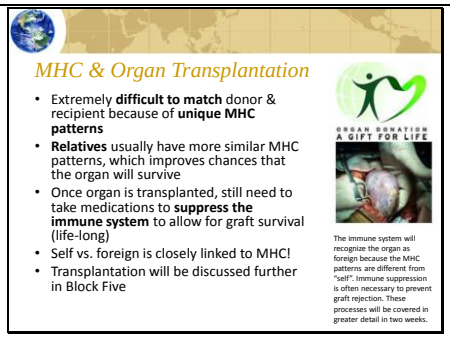
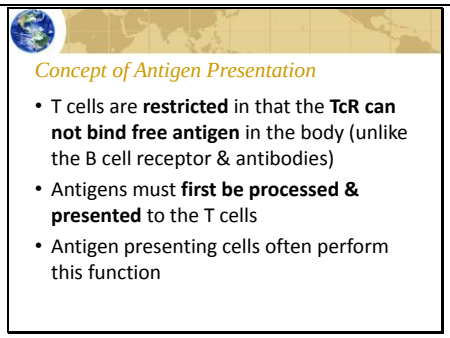
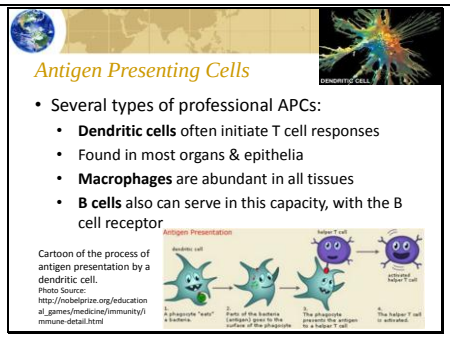
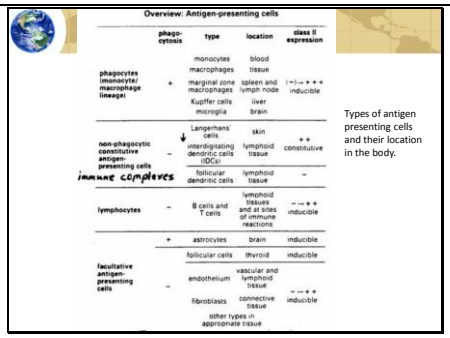
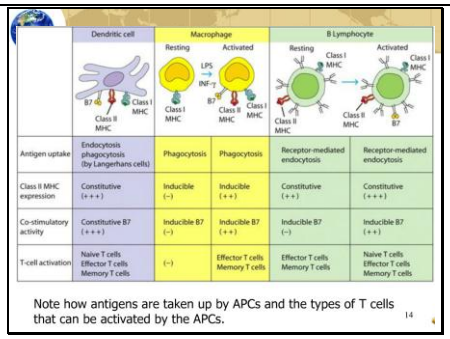
Slide 1	*Foundations of Public Health Immunology* Cell Mediated Immunity: Antigenic Processing, Presentation & Recognition Dendritic cells are important antigen presenting cells that stimulate lymphocytes to generate a cell-mediated response. 	SLIDE 1 Antigen Processing, Presentation & Recognition. This week's lecture will describe how T cells recognize antigen in context of major histocompatibility molecules, with important accessory molecules that serve to control T cell activation and prevent autoimmune reactions. T cell activation must be tightly regulated, as it is difficult to slow down the cell- mediated immune response once it has started. Several cell types are involved, cytokine production increases, and activated T cells have the ability to target and kill other cells. The immune system has developed a system of checks that prevent T cells from deciding on their own what antigens to destroy. Consequently, it is necessary for other cells to process and then present foreign antigens to the T cells with additional signals required for recognition and full activation of the cell- mediated response.
Slide 2	*Objectives* • Identify and explain the locations and functions of MHC molecules. • Identify and explain antigen processing and presentation. • Identify and explain antigen recognition and T-cell selection. • Identify the characteristics of T-dependent and T-independent antigens (including superantigens). • Identify the similarities and differences of T-dependent and T-independent antigens.	SLIDE 2 Learning objectives for Week Eleven: Antigen Processing, Presentation, and Recognition.
Slide 3	*Key Players* • T cells • Antigens • Antigen Presenting Cells (APCs) • Your body's non-immune cells *Key Functions* • MHC, TcR • Antigen processing • Antigen presentation • Antigen recognition T cells (shown in green) clustered in the lymph node. 	SLIDE 3 There are Several components that are necessary for a T cell mediated immune response. These include T cells, antigens, Major Histocompatibility Complex (MHC) proteins, and antigen presenting cells. All of these components have a role in processing, presentation, and recognition of antigens to T cells.

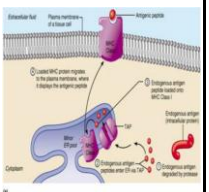
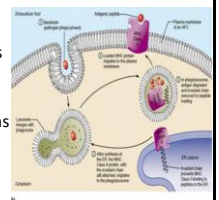
Slide 4		SLIDE 4 In order to present antigens to T cells, Several immune ingredients must come together: antigen, MHC molecules, either "regular" cells or APCs, and T cells.
Slide 5		SLIDE 5 This week we will discuss the T cell mediated immune response to antigens. Once an antigen enters the body, it will be captured and presented to T cells. T cells are extremely important to create an effective adaptive response to a pathogen, especially as CD4 T cells help B cells and the humoral immune response. We will discuss the importance of antigens that are processed and presented to T cells later in the presentation, as this mechanism is the basis for cell- mediated immunity. Remember that there is an epithelial cell lining that protects most of the body, including the skin, mucosal & gastrointestinal tracts. lf a pathogen enters the body through this route, then antigen presenting cells that are present in the area will capture the microbe and process it to display pieces of the protein antigen on its surface. These antigenic peptides are displayed with MHC molecules. Next, these APCs migrate to the lymphoid organs draining the site of infection, where they come into contact with T cells that will recognize the antigen- MHC complex. The T cells will then initiate a cell-mediated immune response.

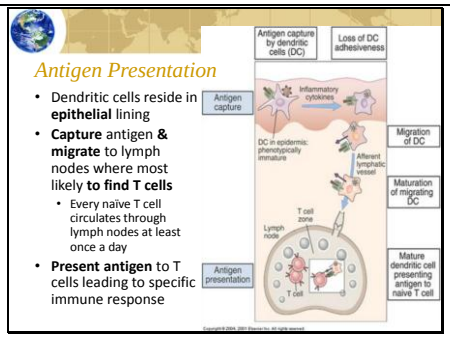
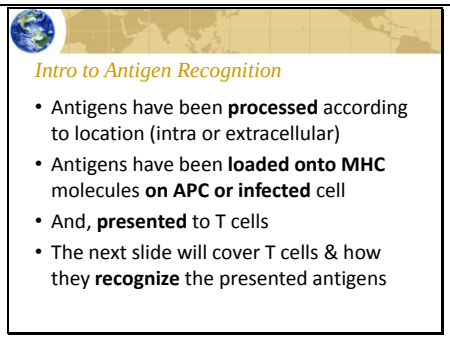
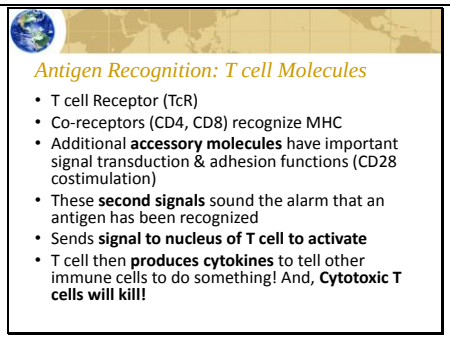
Slide 6		SLIDE 6 Major Histocompatibility Complex (MHC) is a membrane bound protein that functions to display antigen peptides to T cells. The genes that encode these proteins are highly polymorphic, meaning that no person has the exact same MHC as another person (except identical twins). In people, these molecules are more accurately called human leukocyte antigens (HLA), which is important in tissue typing to match transplant organs, etc. The human MHC molecule is located on chromosome 6. The Class I gene complex contains three major loci, B, C and A, and the Class II gene complex contains at least three loci, DP, DQ and DR, which are important for tissue typing and matching of proteins. These genes are found in all mammals, but not in microorganisms. Consequently, the immune system uses these MHC proteins to recognize cells in the body as “self".
Slide 7		SLIDE 7 MHC genes are inherited as a group, one from each parent. The major locus of an MHC gene codes for a polypeptide, the alpha-chain which binds antigen peptides, and is polymorphic (many alleles). Alleles for MHC genes have co-dominant expression, meaning that products (polypeptide) of both parental genes are expressed on the cell surface. The high amount of polymorphism found in MHC genes is thought to confer evolutionary protection for the continuation of a species. Since not everyone in a species will be able to bind certain antigenic peptides or mount a good T cell response to fight an infection, it is likely that someone else in the species will be able to because of different MHC molecules. So, although an individual may not possess MHC molecules capable of binding certain antigenic peptides against a virulent organism, the likelihood is great that other MHC molecules in the species can do so & will prevent the extermination of a species.

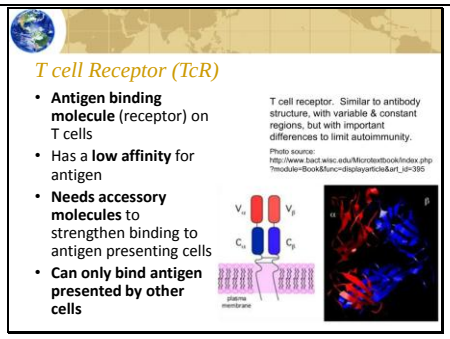
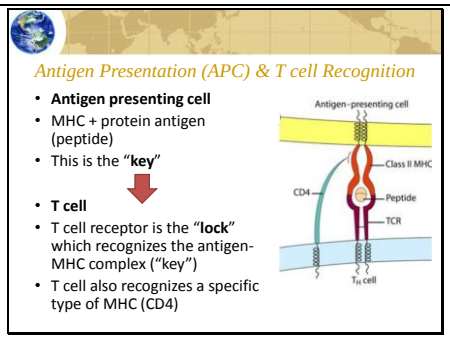
Slide 8		SLIDE 8 Not all cells express both MHC Class I and Class II antigens. MHC Class I molecules are expressed on all nucleated cells and platelets (mature human red blood cells are not protected). MHC Class ll molecules are expressed on only a few cell types, such as dendritic cells, B cells, some macrophages and monocytes, skin associated (Langerhans) cells, and occasionally on other cells. MHC molecules are recognized by separate sets of T cells. Class I MHC molecules are recognized only by cytotoxic T cells (CD8+). Class II MHC molecules are recognized only by helper T cells (CD4+). Mature T cells respond to foreign antigens, but not self antigens. This is another level of control of immune responses. Cytokines can increase the level of expression of class I and class II MHC molecules on antigen presenting cells to capture more antigens (inducible expression).
Slide 9		SLIDE 9 T-cells recognize antigen in association with the major histocompatibility (MHC) molecules. MHC II is a recognition signal between antigen-presenting cells and T-helper cells. In contrast, MHC I is used by T cytotoxic cells for recognition. T cells are restricted so that they can only see antigens presented to them by a specific type of APC that displays either Class I or Class II MHC molecules.

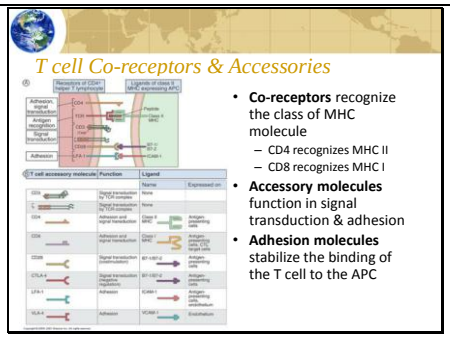
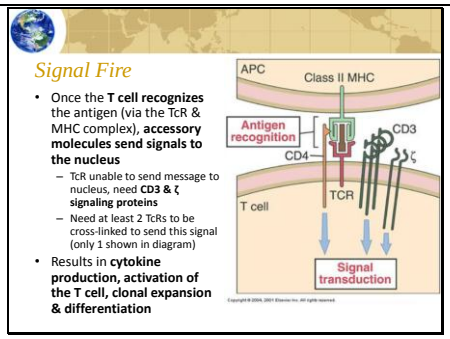
Slide 10	 *MHC & Organ Transplantation* • Extremely **difficult to match** donor & recipient because of **unique MHC patterns** • **Relatives** usually have more similar MHC patterns, which improves chances that the organ will survive • Once organ is transplanted, still need to take medications to **suppress the immune system** to allow for graft survival (life-long) • Self vs. foreign is closely linked to MHC! • Transplantation will be discussed further in Block Five The immune system will recognize the organ as foreign because the MHC patterns are different from "self". Immune suppression is often necessary to prevent graft rejection. These processes will be covered in greater detail in two weeks.	SLIDE 10 Transplant rejection is primarily mediated by T cells. Cytotoxic T cells can directly kill the “nonself" cells that are in the graft or organ, and helper T cells contribute to rejection by secreting cytokines that activate B cells and neutrophils. Unless you have a donor organ from your identical twin, the following problems can occur with transplants: transplanted organs express donor MHC antigens that can be recognized by your immune system. Transplanted organs can then be attacked by your humoral immune response against ABO blood group antigens, vascular endothelial antigens, and histocompatibility (HLA) antigens by your T cells.
Slide 11	 *Concept of Antigen Presentation* • T cells are **restricted** in that the **TcR can not bind free antigen** in the body (unlike the B cell receptor & antibodies) • Antigens must **first be processed & presented** to the T cells • Antigen presenting cells often perform this function	SLIDE 11 Unlike B cells & antibodies, T cells cannot recognize antigen floating free in solution. Consequently, antigens have to first be presented to T cells (antigen presentation) before the T cell can notice the foreign antigen. It is this additional step that helps to regulate the T cell response and prevents T cells from deciding on their own to kill other cells. There are professional cells in the body that function in this capacity- appropriately named antigen presenting cells (such as dendritic cells, macrophages, etc). However, any infected cell in the body can also act like an APC to attract the notice of T cells that they have been hijacked by a virus or bacteria.

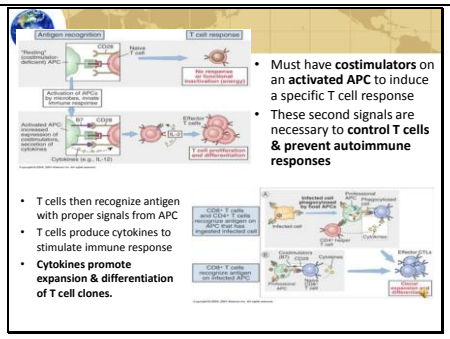
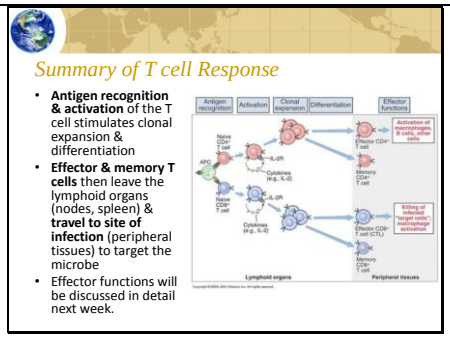
Slide 12	 	SLIDE 12 Antigen presenting cells are extremely important to cell-mediated immunity, as they send the first signal to T cells that a foreign organism has been spotted. Dendritic cells are located throughout the body, and serve as professional antigen presenting cells They can be found in skin, the gastrointestinal tract, and in organs, where they patrol the body for foreign antigens. Once an APC captures an antigen, it migrates to the draining lymph nodes nearest to the site of capture (infection) where it “presents” its antigen to T cells.
Slide 13	 	SLIDE 13 This table lists some types of antigen presenting cells and where they are located in the body. Note that monocytes function as APCs in the blood, whereas macrophages are located in tissues. In addition, Langherhans' cells are a type of dendritic cell located in the skin. Remember, dendritic cells are the best antigen presenting cell type that can also influence the T cell & the type of immune response that is produced to an antigen.
Slide 14	 	SLIDE 14 Table showing how antigens are taken up and processed by APCs and the types of T cells that are subsequently activated. Activated macrophages can induce higher expression of MHC II molecules on their surface to present more antigens on their surface to T cells to stimulate a stronger response.

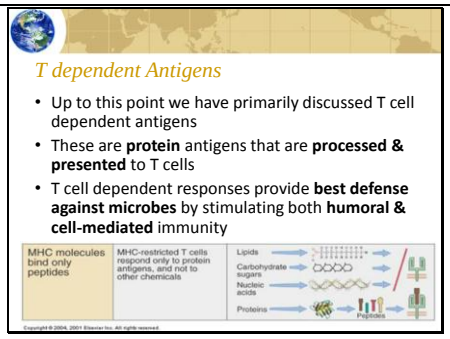
Slide 15	*Antigen Processing* • Now have MHC and APC • But, how does the antigen fit in?? • **Antigens will be displayed by the MHC** molecules located on the surface of the APC (similarly for other cells in the body) • Antigens are **processed 2 ways**	SLIDE 15 Antigens must first be processed before they can be presented on the surface of the cell to T cells. The entire antigen will not be presented to a T cell; instead a protein antigen will be "processed" so that small peptides are complexed with MHC molecules on the surface of the cell. Antigens are processed in two ways, through the endogenous or exogenous pathways. The pathway that is chosen depends on if the foreign microbe has infected a normal host cell (e.g. a virus that lives inside the cell) or if it has been phagocytosed by an antigen presenting cell (like a macrophage) from outside of the cell.
Slide 16	*Antigen Processing: Intracellular* • **Intracellular** antigens • Endogenously synthesized Ag within **target cell** proteasomes associate with **Class I** proteins • The MHC I & foreign protein are then inserted into target cell membrane • **Viral antigens and tumor cells** are presented this way! 	SLIDE 16 Alter a cell has degraded endogenous antigen (intracellular) within its proteasome, it associates this antigen with a Class I protein then inserts it into its membrane. Afterwards, cytotoxic T cells normally respond to this antigen.
Slide 17	*Antigen Processing: Extracellular* • **Extracellular** antigens • Processed **inside a macrophage**, exogenous Ag is broken down into peptide fragments in phagolysome, and then bound to **Class II** proteins on the APC cell surface • **Bacterial and parasitic antigens** are often processed this way 	SLIDE 17 Alter phagocytosis, exogenous antigen (extracellular) is broken down into peptide fragments in a phagolysome and then bound to MHC Class 2 proteins on the antigen- presenting cell surface. TH2 helper cells then respond to this antigen.

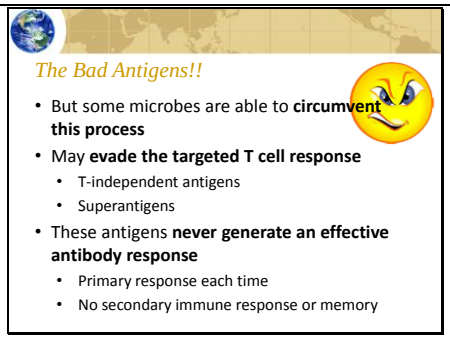
Slide 18		SLIDE 18 Dendritic cells are extremely important antigen presenting cells because they are the most potent activators of native T cells. They can also influence the nature of the response once they activate the T cell by directing a CD4+ response against certain microbes. An amazing property of antigen presentation is that if an antigen is introduced anywhere in the body, a T cell response begins in the lymph nodes draining that site within 12 to 18 hours!
Slide 19		SLIDE 19 The next part of the presentation describes antigen recognition. Antigen recognition is how the T cell, the TCR, & associated co- receptors bind to the antigen presenting cell (via MHC molecules) to "see" the antigen. T cells must make cell to cell contact with antigen presenting cells because MHC molecules are membrane-associated & not soluble.
Slide 20		SLIDE 20 This slide emphasizes the elements that must be present to have antigen recognition, as the T cell receptor and accessory molecules have important functions to activate the T cell. A brief review from last week: The T cell receptor (TcR) is critical for antigen recognition because it interacts with the MHC molecules complexed to peptide antigens. Coreceptors are important for recognition of MHC molecules (CD4-MHC II or CD8-MHC I), and are functionally segregated into helper and cytotoxic cells. In addition, Several accessory molecules are present on the surface of a T cell & antigen presenting cell with important signal transduction and adhesion functions. Once the TcR binds to the MHC +Ag, these accessory molecules are involved in transmitting a signal to the nucleus of the T cell to activate it to either kill the cell or get help, depending on the type of T cell.

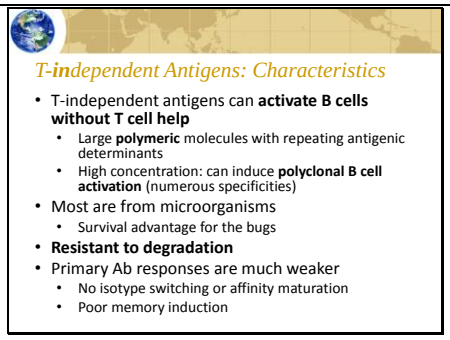
Slide 21		SLIDE 21 The TcR has many similarities to a B cell receptor or antibody. The TcR has tremendous diversity (similar to B cell Fab regions) as coded by variable gene segments V, D, J & C. Unlike antibodies, the TcR is found only as a cell surface molecule, and is not secreted which has made it more difficult to study. But, there are over 30,000 receptors per cell!! The TcR cannot recognize antigens in their native state- antigens must be processed first & presented. In addition, the T cell receptor does not undergo somatic hypermutation like the B cell receptor (antibodies) because it does not need a high level of variability (diversity) to bind to the MHC proteins on antigen presenting cells or infected host cells. This is an important concept because it does not allow the T cell to change (through hypermutation) its TCR after negative selection has occurred in the thymus. One of many checks & balances to prevent autoimmune reactions!
Slide 22		SLIDE 22 Antigen presentation is analogous to a key (MHC + Ag) on an antigen presenting cell turning the lock (T cell receptor) on a T cell to release it for action.

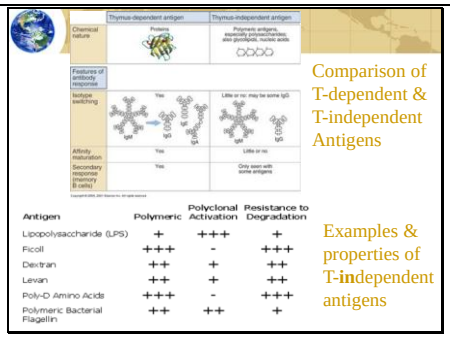
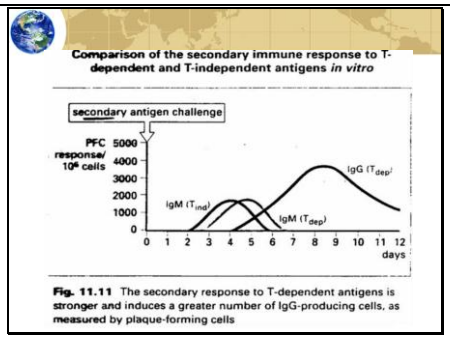
Slide 23		SLIDE 23 In Figure A, the T cell is on the left side of the diagram. Note the accessory molecules such as the CD4 co-receptor that recognizes the MHC II molecule. Then on the right side of the diagram, the antigen presenting cell has processed the antigen that it has found in the body & is presenting it to the T cell to create a response to that specific antigen. Note the functions of the additional accessory molecules that improve adhesion or signal to other cells to regulate the immune response. Adhesion molecules stabilize the MHC - TcR complex and allow for antigen recognition. Figure B describes the various types of accessories on both the T cell and antigen presenting cell that are needed to activate a response. The binding of the TcR with the antigen peptide-MHC complex delivers the first signal to the T cell. However, this is not enough to activate the T cell. A second signal is necessary for activation & will be described further on the next slides.
Slide 24		SLIDE 24 CD3 complex is a group of proteins that are physically associated with the TcR. The CD3 complex functions to ensure the cell surface expression of the TcR, as well as to send activating signals to the interior of the T cell once antigen is bound. These signals include tyrosine kinases and transcription factors that function to increase expression of certain genes in the nucleus of the T cell. The transcription of these genes initiate growth and proliferation of the T cell, and cytokine production.

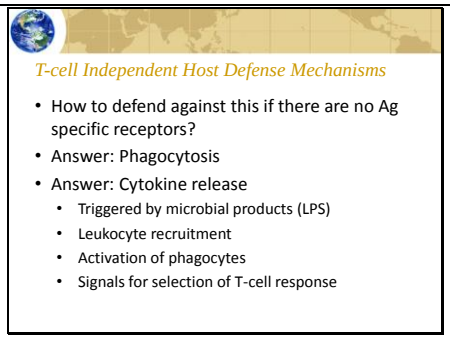
Slide 25	 	SLIDE 25 The binding of a T cell to an antigen presenting cell (APC) is by itself not enough for T cell activation that tums it into an effector cell. Costimulatory molecules must also bind to deliver a second signal required for T cell activation. The most important costimulatory molecule on the T cell is CD28, which binds to either B7-1 (CDBO) or B7-2 (CD86). T cells are constantly on patrol throughout the body. They check the MHC molecules of all cells to see if it has bound antigen In most cases, no antigen is bound, but the T cells “see” the self antigens, or MHC molecules on the surface of cells. So, although T cells may encounter these self antigens in the body, they will not respond unless they receive a second signal that causes them to recognize the antigen as foreign. In fact, binding of their TCR (signal one) without signal two causes them to self- destruct by apoptosis. After they receive both signals, they differentiate into effector cells that can either kill the APC (if it is a CD8+ cytotoxic T cell), carry out cell-mediated immune responses (if it is a CD4+ Th1 cell), or provide help to B cells (if it is a CD4+ Th2 cell).
Slide 26	 	SLIDE 26 Like B cells, T cells undergo clonal expansion and differentiation alter antigen recognition. Native T cells recognize antigen and costimulators from APCs in lymphoid organs (nodes, spleen, etc), which activate the T cell to expand (by cytokines) and differentiate into effector and memory cells. These differentiated T cells then leave the lymphoid organs and migrate to the peripheral tissues and the site of infection. Once there, they perform specific effector functions to target the microbe.

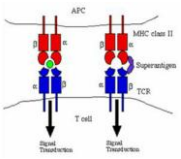
Slide 27	 *T dependent Antigens* • Up to this point we have primarily discussed T cell dependent antigens • These are **protein** antigens that are **processed & presented** to T cells • T cell dependent responses provide **best defense against microbes** by stimulating both **humoral & cell-mediated** immunity MHC molecules bind only peptides MHC-restricted T cells respond only to protein antigens, and not to other chemicals Lipids Carbohydrate sugars Nucleic acids Proteins Peptides	SLIDE 27 T dependent antigens are antigens that are first processed & then presented to T cells, as we have detailed in the lecture so far. Antigen presentation to T cells provides the best response to microbes, as it stimulates both humoral and cell-mediated immunity. But, this is not always the case!! Remember, antigens that produce a strong immune response typically are protein-based, and are more potent & generate the best immune response because they are presented to T cells.

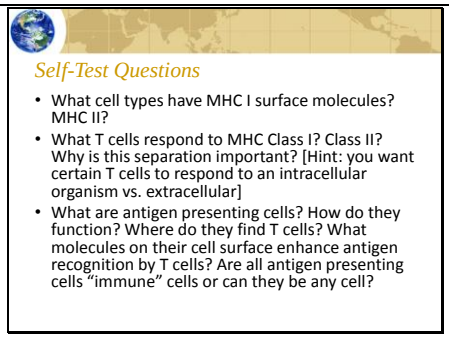
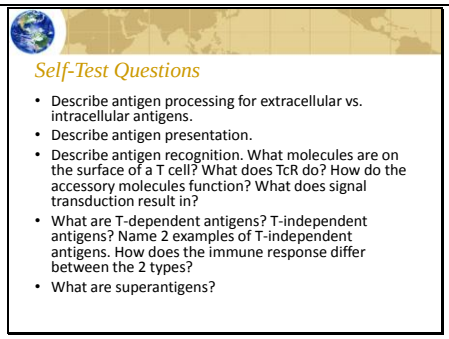
Slide
 	
28
	
	The Bad Antigens!!
	• But some microbes are able to **circumvent this process**
	• May **evade the targeted T cell response**
	  • T-independent antigens
	  • Superantigens
	• These antigens **never generate an effective antibody response**
	  • Primary response each time
	  • No secondary immune response or memory
		SLIDE 28 T-independent antigens can activate B-cells without T-cell assistance. The primary antibody response to these antigens is normally rather weak and does not produce isotype switching or affinity maturation In addition, there is minimal memory induction produced by these antigens. This means that there is no secondary antibody response produced with T- independent antigens.

Slide
 	
29
	
	T-independent Antigens: Characteristics
	• T-independent antigens can **activate B cells without T cell help**
	  • Large **polymeric** molecules with repeating antigenic determinants
	  • High concentration: can induce **polyclonal B cell activation** (numerous specificities)
	• Most are from microorganisms
	  • Survival advantage for the bugs
	• **Resistant to degradation**
	• Primary Ab responses are much weaker
	  • No isotype switching or affinity maturation
	  • Poor memory induction
		SLIDE 29 T-independent antigens are normally large highly resistant polymeric molecules with repeating antigenic determinants. In sufficient concentrations, these antigens can induce polyclonal B-cell activation. These antigens are from microorganisms and may provide a survival advantage for the microbes. These characteristics may protect the microbe by causing the immune system to not generate secondary antibody responses & forget the organism over time.

Slide 30	 Comparison of T-dependent & T-independent Antigens Examples & properties of T-independent antigens	SLIDE 30 This slide provides examples of T-independent antigens (lipopolysaccharide [LPS], bacterial flagellin, etc.) and their characteristics. Note that isotype switching does occur with T dependent antigens, but not with T independent antigens.
Slide 31	 Comparison of the secondary immune response to T-dependent and T-independent antigens *in vitro* secondary antigen challenge PFC response/ 10^6 cells IgM (T_{ind}) IgG (T_{dep}) IgM (T_{dep}) days **Fig. 11.11** The secondary response to T-dependent antigens is stronger and induces a greater number of IgG-producing cells, as measured by plaque-forming cells	SLIDE 31 This slide compares the immune response of T-dependent and T-independent antigens. Notice that there is no secondary immune response with T-independent antigens therefore, there is no IgG production and no faster immune response.

Slide
 	
32
	
	T-cell Independent Host Defense Mechanisms
	• How to defend against this if there are no Ag specific receptors?
	• Answer: Phagocytosis
	• Answer: Cytokine release
	  • Triggered by microbial products (LPS)
	  • Leukocyte recruitment
	  • Activation of phagocytes
	  • Signals for selection of T-cell response
		SLIDE 32 Cell-mediated immunity involves T-cells which exhibit antigen specificity via the T-cell receptor or TcR. T-cells perform numerous functions including effector functions on macrophages and other T-cells, cell killing via Tc cells, Type 4 delayed hypersensitivity reactions, and interactions with natural killer cells and antigen-dependent cell-mediated cytotoxicity (ADCC). The subsets of T- cells help determine and regulate the cell-mediated response.

Slide 33	*Superantigens* • **Molecules which can activate T cells nonspecifically** • Usually of **bacterial** origin, but also some viral proteins • Examples include Staphylococcal enterotoxins, exofoliative dermatitis toxin, toxic shock syndrome 	SLIDE 33 Superantigens usually nonspecifically activate T-cells. They are normally bacterial components that can bind multiple T-cells and can either induce a stronger or weaker immune response.
Slide 34	*Properties of Superantigens* • **Superantigens are not "processed"** • Different binding process to TcR & MHC class II on antigen presenting cell • Can **bind to many** T cells • Can induce immune response or clonal anergy (like conventional Ag)  Comparison of normal antigen (green) vs. a superantigen (purple) to illustrate the altered binding to the Tcr-MHC complex II. Diagram source: http://www.bio.davidson.edu/Courses/Immunology/Students/spring2000/white/restricted/TSS.html	SLIDE 34 Superantigens are not "processed" by the immune system and have different binding properties from other antigens. These proteins bind to and activate all of the T cells in a person that express a certain set of VB T cell receptor. Superantigens bind to nonpolymorphic regions of the class ll MHC molecules on antigen presenting cells & the VB regions of TcRs. They can activate many T cells at once, resulting in large cytokine production and toxic shock syndrome. Several staph organisms produce enterotoxins that act as superantigens.
Slide 35	*In Summary* • Understand the concepts of antigen processing and presentation • MHC restriction for T cells (e.g. Cd4 recognizes MHC II) • T cell selection • Antigen recognition • TcR, accessory molecules required • "Bad" antigens • T-independent vs. T-dependent immune response	SLIDE 35 What you need to know . . .

Slide 36	 *Self-Test Questions* • What cell types have MHC I surface molecules? MHC II? • What T cells respond to MHC Class I? Class II? Why is this separation important? [Hint: you want certain T cells to respond to an intracellular organism vs. extracellular] • What are antigen presenting cells? How do they function? Where do they find T cells? What molecules on their cell surface enhance antigen recognition by T cells? Are all antigen presenting cells "immune" cells or can they be any cell?	SLIDE 36 Self test questions for antigen processing, presentation, & recognition.
Slide 37	 *Self-Test Questions* • Describe antigen processing for extracellular vs. intracellular antigens. • Describe antigen presentation. • Describe antigen recognition. What molecules are on the surface of a T cell? What does TcR do? How do the accessory molecules function? What does signal transduction result in? • What are T-dependent antigens? T-independent antigens? Name 2 examples of T-independent antigens. How does the immune response differ between the 2 types? • What are superantigens?	