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Immunology 2017: Lecture 8 handout

Antigen presentation

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INTRODUCTION

We know by now that there are two types of specific (adaptive) immunity

1. *Humoral immunity* that is mediated by B lymphocytes and their antibodies
2. *Cellular immunity* which is performed through T cells.

Both, T and B lymphocytes have specific receptors that recognize a complementary antigen (cognate antigen) however there are differences in antigen recognition between these two cells.

B cells **directly** recognize antigens via their receptors. They recognize **peptide as well as polysaccharide , DNA fragments and lipid antigens.** They recognize a small portion of the antigen called an epitope, this makes it possible for them to recognize **linear or conformational** peptides (this means they can recognize a folded protein)On the other hand, T cells can not directly recognize antigens. **The antigen must be processed and presented to T cells.** ***Processed*** means: the antigen must be cut enzymatically to suitable linear peptide chain with a certain length. ***Presented*** means: this processed antigen binds to Major Histocompatibility molecule (MHC) and this MHC molecule with the antigen bound to it must attach to a cell membrane to be recognized to the T cell. Moreover, **T lymphocytes recognize only peptide antigens** only. And these should be **linear** not conformational.

Features of Antigens Recognized by T Cells	Explanation
Most T cells recognize peptides and no other molecules.	Only peptides bind to MHC molecules.
T cells recognize linear peptides and not conformational determinants of protein antigens.	Linear peptides bind to clefts of MHC molecules, and protein conformation is lost during the generation of these peptides.
T cells recognize cell-associated and not soluble antigens.	Most T cell receptors recognize only peptide-MHC complexes, and MHC molecules are membrane proteins that display stably bound peptides on cell surfaces.
$CD4^+$ and $CD8^+$ T cells preferentially recognize antigens ingested from the extracellular environment into vesicles and antigens produced in the cytosol, respectively.	Pathways of assembly of MHC molecules ensure that class II molecules display peptides that are proteolytically degraded in vesicles in APCs and that class I molecules present peptides from cytosolic proteins that are degraded by cytosolic proteasomes.

ANTIGEN PRESENTATION

So how does this antigen presentation happen?

It occurs through two mechanism each serving a different purpose

1. **MHC I molecules** are expressed by *all* cells. They present samples of *intracellular proteins* (endogenous proteins) to *cytotoxic T* cells (these are also called killer T cells or CD8+ T cells) NOTE: do not mix killer T with natural killer, they are completely different

2. **MHC II molecules** are expressed by *certain cells*, mainly dendritic cells, activated macrophages and activated B cells. These process *extracellular* antigens and present them to *T helper* cells (CD 4+ cells)

NOTE: A single T cell can recognize a specific peptide displayed by only one of the large number of different MHC molecules that exist. This phenomenon is called **MHC restriction**

MHC CLASS I

Class 1 MHC have a binding groove that is **closed at both ends**, so the peptides they present must fit within the groove. So they are usually **eight or nine amino acids in length (these numbers vary slightly depending on which book you're reading from)** . These peptides are **anchored at the ends**, and the slight variation in length is accommodated by letting the peptide bulge out a bit in the centre. These peptides must be **linear to fit within the groove**.

Every human has three genes for class I MHC proteins on chromosome 6 (HLA-A, HLA-B, and HLA-C) note that MHC are the same as HLA = human leukocyte antigens.

Because we have two chromosomes 6's (one maternal and one paternal), we have a total of six class I MHC genes. Each class I HLA protein pairs with another protein called β2-microglobulin to make up the complete class I MHC molecule. In the human population, there are about 1500 slightly different forms of the genes that encode the three class I HLA proteins. The proteins encoded by the variants of the HLA-A, HLA-B, and HLA-C genes have roughly the same shape, **but they differ by one or a few amino acids. So HLA molecules are polymorphic which means they molecules that have many forms which are slightly different between individuals.**

Note: we have the same gene for the β2-microglobulin protein, it is not polymorphic.

Because they are polymorphic, class I MHC molecules can have different binding motifs (sites), and therefore can present peptides which have different kinds of amino acids at their ends. For example, some class I MHC molecules bind to peptides that have hydrophobic amino acids at one end, whereas other MHC molecules prefer basic amino acids at this anchor position. Since humans have the possibility of expressing up to six different class I molecules, **collectively our class I molecules can present a wide variety of peptides**. Moreover, although MHC I molecules are picky about binding to certain amino acids at the ends of the peptide, they do not select the amino acids at the center of the protein fragment. As a result, a given class I MHC molecule can bind to and present many different peptides, each of which "fits" with the particular amino acids present at the ends of its binding groove.

Class 1 MHC bind to endogenous proteins. Which means the proteins synthesized in the cell as well as proteins encoded by viruses and other parasites that may have infected the cell. For example, when a virus enters a cell, it uses the cellular biosynthetic machinery to produce proteins encoded by viral genes. A sample of these viral proteins is then displayed by class I MHC molecules along with samples of all the normal cellular proteins.

CD 8 lymphocytes inspect the protein fragments displayed by class I MHC molecules. Consequently, almost every cell is an "open book" that can be checked by these cytotoxic T cells to determine whether it has been invaded by a virus or other parasite and should be destroyed.

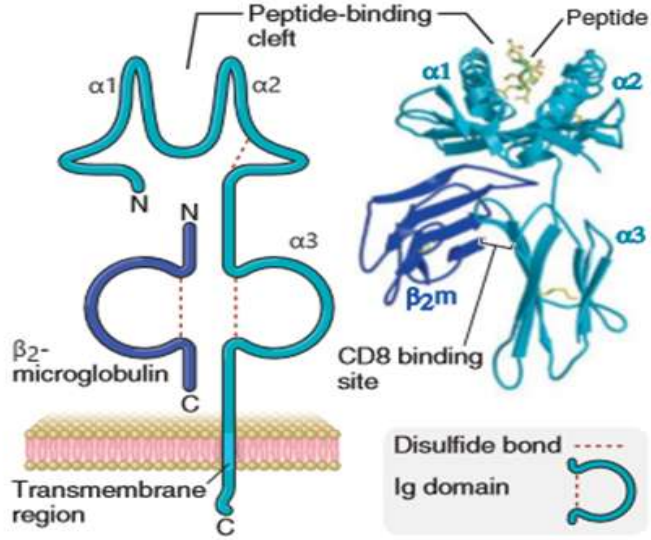

FIGURE 6.9 Structure of a class I MHC molecule. The schematic diagram *(left)* illustrates the different regions of the MHC molecule (not drawn to scale). Class I molecules are composed of a polymorphic α chain noncovalently attached to the nonpolymorphic β_2-microglobulin (β_2m). The α chain is glycosylated; carbohydrate residues are not shown. The ribbon diagram *(right)* shows the structure of the extracellular portion of the HLA-B27 molecule with a bound peptide, resolved by x-ray crystallography. *(Courtesy of Dr. P. Bjorkman, California Institute of Technology, Pasadena.)*

ANTIGEN PROCESSING FOR MHC 1 BINDING

When mRNA is translated into protein in the cytoplasm, mistakes are frequently made resulting in abnormally folded, useless proteins. These are cut by proteasomes into peptides. Most of these peptides are then broken down further into individual amino acids, which are reused to make new proteins. However, some of the peptides created by the proteasomes are carried by specific **transporter proteins (TAP1 and TAP2)** across the membrane of the endoplasmic reticulum (ER) .TAP transporter has the highest affinity for peptides that are 8–16 amino acids long. Consequently, the TAP transporter screens peptides produced by proteasomes, and preferentially transports those that have the right kinds of C-termini and which are approximately the correct length. **Once class I MHC molecules are loaded with peptides, they proceed to the surface of the cell for display.**

SUMMARY OF PRESENTATION WITH TYPE1 MHC

-MHC 1 are present in all cells including the specialized antigen presenting cells.

-MHC 1are the same as HLA. They are encoded by a gene locus on chromosome 6

-There are 6 HLA proteins in each individual two HLA-A (one paternal and one maternal), 2 HLA-B and 2 HLA-C

-Because both maternal and paternal genes are expressed, the genes are codominant

-There is a huge polymorphism in these HLA proteins allowing for recognition of variable antigens

-each HLA protein chain is attached to a beta 2 microglobulin chain which has no polymorphism

-HLA 1 has a binding groove that is closed at both ends. So, peptide antigens attached to it must be of a specific length (usually 8-9 amino acids)

- HLA bind to endogenous antigens derived from normal cellular proteins or viruses and parasites that infected the cell (entered inside the cell)

-these endogenous proteins are cut randomly by the proteasome to peptide fragments.

-these fragments are transported by TAP proteins across the ER.

-fragments which fit within HLA binding site and have the correct amino acid at the ends will attach to the HLA

-The HLA and attached peptide are then expressed on the surface of the cell.

-T cell receptors on CD8 cells can recognize these processed antigens. If they recognize self-protein they do not react, but if they recognize viral protein they kill the infected cell

NOTE: Please remember that Natural killer cells also recognize MHC1 via their inhibitory receptors.

CLASS II MHC MOLECULES

Like class I molecules, the class II MHC molecules (encoded by genes in the HLA-D region of chromosome 6) are wildly **polymorphic**. Within the human population, there are about 700 different versions of the class II MHC molecules.

There are **three class II HLA gene loci called HLA-DP, HLA-DQ, and HLA-DR**. Each class II MHC molecule is composed of a heterodimer **of α and β polypeptides**. The DP, DQ, and DR loci on each chromosome contain separate genes designated A and B, encoding the α and β chains, respectively. Every individual has two HLA-DP genes (called DPA1 and DPB1), two HLA-DQα genes (DQA1, 2), one HLA-DQβ gene (DQB1), one HLA-DRα gene (DRA1), and one or two HLA-DRβ genes (DRB1 and DRB3, 4, or 5). (don't worry too much about these details, just get the idea that there are several type II proteins in each individual and that these are polymorphic)

The set of MHC alleles present on each chromosome is called an MHC haplotype. For instance, an HLA haplotype of an individual could be HLA-A2, B5, DR3, and so on . All heterozygous individuals, of course, have two HLA haplotypes. **individuals will usually express all of the MHC alleles in the two haplotypes inherited from their parents (codominance).**

In contrast to class I MHC molecules, the binding groove of class II MHC molecules is open at both ends, so a peptide can hang out of the groove. So, peptides that bind to class II molecules are longer than those that occupy the closed groove of class I molecules – in the range of 13–25 amino acids. Further, for class II MHC molecules, the critical amino acids that anchor the peptides are spaced along the binding groove instead of being clustered at the ends

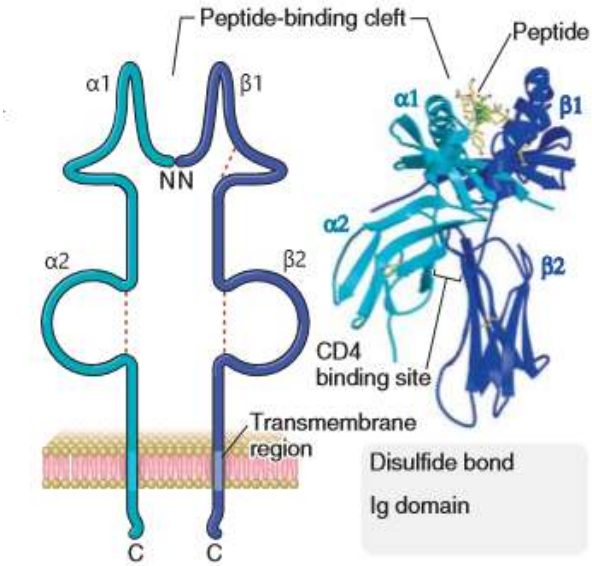

FIGURE 6.11 Structure of a class II MHC molecule. The schematic diagram *(left)* illustrates the different regions of the MHC molecule (not drawn to scale). Class II molecules are composed of a polymorphic α chain noncovalently attached to a polymorphic β chain. Both chains are glycosylated; carbohydrate residues are not shown. The ribbon diagram *(right)* shows the structure of the extracellular portion of the HLA-DR1 molecule with a bound peptide, resolved by x-ray crystallography. *(Courtesy of Dr. P. Bjorkman, California Institute of Technology, Pasadena.)*

ANTIGEN PRESENTATION BY CLASS II MHC

class II MHC molecules are made up of two proteins (called the **α and β chains**) which are produced in the cytoplasm and are injected into the endoplasmic reticulum where they bind to a third protein called the **invariant chain**. This invariant chain protein sits in the groove of the MHC II to prevent it from binding to peptides in the ER . It also guides MHC II through the Golgi apparatus to special vesicles in the cytoplasm called the **endosome. It is within endosomes that class II MHC molecules are loaded with peptides.**

proteins that are present outside the APC are enclosed in a **phagosome**, and brought into the cell. **This phagosome then merges with the endosome**, and **enzymes present in the endosome cuts the exogenous proteins into peptides.** Note that cutting of exogenous antigens within APC is done via specific enzymes like **LMP2, LMP7, and MECL1 and** not via proteasome.

During this time, endosomal enzymes also destroy all of the invariant chain except for the piece called **CLIP** that actually is guarding the groove of the MHC molecule.

Although the exogenous proteins and the invariant chain are cut by enzymes in the endosome, the class II MHC molecule itself is not cut by these enzymes. This is presumably because the MHC molecule is cleverly **folded so that the enzymes cannot gain access to their cleavage sites**. Meanwhile, a cellular protein, HLA-DM, which also has travelled to the endosome, catalyses the release of CLIP, allowing an exogenous peptide to be loaded into the now-empty groove of the class II MHC molecule. But HLA-DM does more than just kick CLIP out to make room for the peptide. HLA-DM competes with potential peptides for binding to the class II MHC molecule, insuring that only peptides that bind tightly will be presented. Finally, the complex of MHC plus peptide is transported to the cell surface for display and be recognized by CD4 cells.

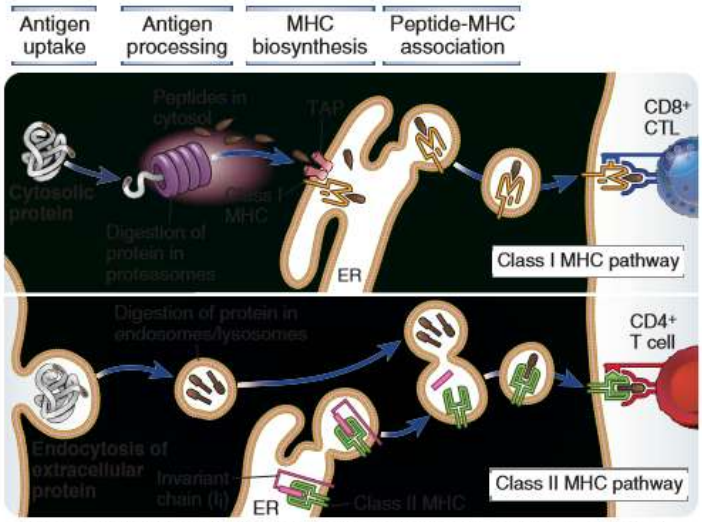

FIGURE 6.13 Pathways of antigen processing and presentation. In the class I MHC pathway *(top panel)*, protein antigens in the cytosol are processed by proteasomes, and peptides are transported into the endoplasmic reticulum (ER), where they bind to class I MHC molecules. In the class II MHC pathway *(bottom panel)*, protein antigens that are degraded in lysosomes bind to class II MHC molecules. Details of these processing pathways are shown in Figs. 6.14 and 6.15. *ER*, endoplasmic reticulum; *TAP*, transporter associated with antigen processing.

TABLE 6.4 Comparative Features of Class I and Class II MHC Pathways of Antigen Processing and Presentation

Feature	Class I MHC Pathway	Class II MHC Pathway
Composition of stable peptide-MHC complex	Polymorphic α chain, β_2-microglobulin, peptide 	Polymorphic α and β chains, peptide 
Types of APCs	All nucleated cells	Dendritic cells, mononuclear phagocytes, B lymphocytes, endothelial cells, thymic epithelium
Responsive T cells	$CD8^+$ T cells	$CD4^+$ T cells
Site of antigen degradation	Proteasome	Late endosomes and lysosomes
Source of protein antigens	Mainly cytosolic proteins (usually synthesized in the cell; may enter cytosol from phagosomes); also nuclear and membrane proteins	Endosomal and lysosomal proteins (mostly internalized from extracellular environment)
Enzymes responsible for protein degradation	β1, β2, and β5 subunits of proteasomes	Endosomal and lysosomal proteases (e.g., cathepsins)
Site of peptide loading of MHC	Endoplasmic reticulum	Late endosomes/lysosomes
Molecules involved in transport of peptides and loading of MHC molecules	TAP in ER	Invariant chain in ER, Golgi; DM

APC, Antigen-presenting cell; *ER*, endoplasmic reticulum; *MHC*, major histocompatibility complex; *TAP*, transporter associated with antigen processing.

Comparison between MHC I & II

Feature	Class I MHC	Class II MHC
Polypeptide chains	α and β_2-microglobulin	α and β
Locations of polymorphic residues	α1 and α2 domains	α1 and β1 domains
Binding site for T cell coreceptor	CD8 binds mainly to the α3 domain	CD4 binds to a pocket created by parts of α2 and β2 domains
Size of peptide-binding cleft	Accommodates peptides of 8–11 residues	Accommodates peptides of 10–30 residues or more
Nomenclature		
Human	HLA-A, HLA-B, HLA-C	HLA-DR, HLA-DQ, HLA-DP

ANTIGEN PRESENTING CELLS

the term "antigen presenting cell" always refers to **those special cells** which can provide both the **high levels of MHC proteins** and **co-stimulatory molecules** required for T cell activation

Co-stimulation usually involves a protein called B7 on the surface of an antigen presenting cell which acts with CD28 on the surface of the T cell.

There are three types of antigen presenting cells:

1.*activated dendritic cells*

2.*activated macrophages*

3.*activated B cells.*

	Expression of		
Cell Type	**Class II Major Histocompatibility Complex**	**Costimulators**	**Principal Function**
Dendritic cells	Constitutive; increases with maturation; increased by IFN-γ and T cells (CD40L-CD40 interactions)	Constitutive; expression is increased with TLR signals, IFN-γ, CD40-CD40L interactions	Antigen presentation to naive T cells in initiation of T cell responses to protein antigens (priming)
Macrophages	Low or negative; increased by IFN-γ and T cells (CD40L-CD40 interactions)	Expression is increased by TLR signals, IFN-γ, CD40-CD40L interactions	Antigen presentation to effector $CD4^+$ T cells in effector phase of cell-mediated immune responses (T cell–enhanced killing of phagocytosed microbes)
B lymphocytes	Constitutive; increased by IL-4, antigen receptor cross-linking, and T cells (CD40L-CD40 interactions)	Expression is increased by T cells (CD40-CD40L interactions), antigen receptor cross-linking	Antigen presentation to $CD4^+$ helper T cells in humoral immune responses (helper T cell–B cell interactions)

***NOTE**: Microbes and protein antigens that enter through epithelia are concentrated in lymph nodes, and dendritic cells are the cells that are best able to capture, transport, and present antigens to T cells.*

Antigens that enter the bloodstream may be sampled by DCs that are resident in the spleen, or captured by circulating (mostly plasmacytoid) DCs and taken to the spleen. See pic next page:

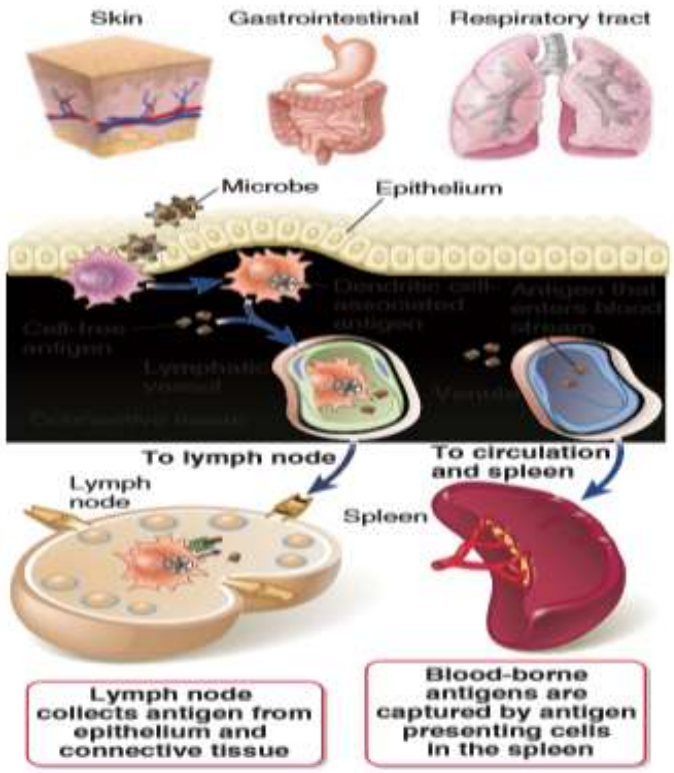

FIGURE 6.3 Routes of antigen entry. Microbial antigens commonly enter through the skin and gastrointestinal and respiratory tracts, where they are captured by dendritic cells and transported to regional lymph nodes. Antigens that enter the bloodstream are captured by APCs in the spleen.

DENDRITIC CELLS

Activated dendritic cells Dendritic cells (DCs) have a characteristic, starfish-like shape, and get their name from the word “dendrite”. These cells are the most important of all the antigen presenting cells – because dendritic cells can initiate the immune response by activating virgin T cells.

Please note that these cells are very different from the plasmacytoid dendritic cells (pDCs) whose primary function is to produce large amounts of interferon α and β in response to a viral attack. In fact, pDCs are not even shaped like a starfish. They are round, like plasma cells. refer to lecture 2 when we talked about interferons and viral infection.however these can also act as antigen presenting cells especially for blood borne infections.

The first DCs described were starfish-shaped, “Langerhans” cells that are found in the tissues just below the skin. However, dendritic cells have since been discovered all over the body they take up positions beneath the barriers of epithelial cells that represent our first line of defence.

In this “resting” state, DCs express some **B7** and relatively **low levels of MHC** molecules on their surface. As a result, these resting dendritic cells are not very good at presenting antigen to T cells, especially to virgin T cells. This is because naive T cells require extensive receptor crosslinking by MHC–peptide complexes as well as powerful co-stimulation to be activated.

Dendritic cells are activated when they recognize a pathogen through their toll like receptors. This antigen enters the dendritic cell via phagocytosis. These peptide antigens are processed by specific

enzymes within the dendritic cell to peptides suitable to attach to the binding groove of class II MHC molecules. The dendritic cell as it matures after activation migrates from its site beneath the skin to reach the lymph nodes where T cells are found. During this journey DC increases MHC 1 & II production.

Also while traveling, the DC increases production of B7 co-stimulatory proteins. So when it reaches a lymph node, the mature dendritic cell has everything it needs to activate virgin T cells: **high levels of class I and class II MHC molecules loaded with the appropriate peptides, and plenty of B7 proteins**

Once a DC reaches a lymph node, it only lives for about a week. This short lifetime may seem strange, However, dendritic cells can interact with hundreds or even thousands of T cells every hour, and their short presentation life insures that dendritic cells carry snapshots of the battle which are up-to-date. In addition, after a dendritic cell has been activated, but before it begins its travels, it produces **special cytokines (chemokines**) which encourage monocytes to leave the blood, enter the tissues, and become dendritic cells. Consequently, ***activated dendritic cells recruit their own replacements.*** These newly recruited DCs can carry fresh images of the battle to lymph nodes as the battle continues.

Once DCs reach the lymph node they activate T cells which exit the lymph nodes, circulate through the blood, and enter inflamed tissues to help with the battle.

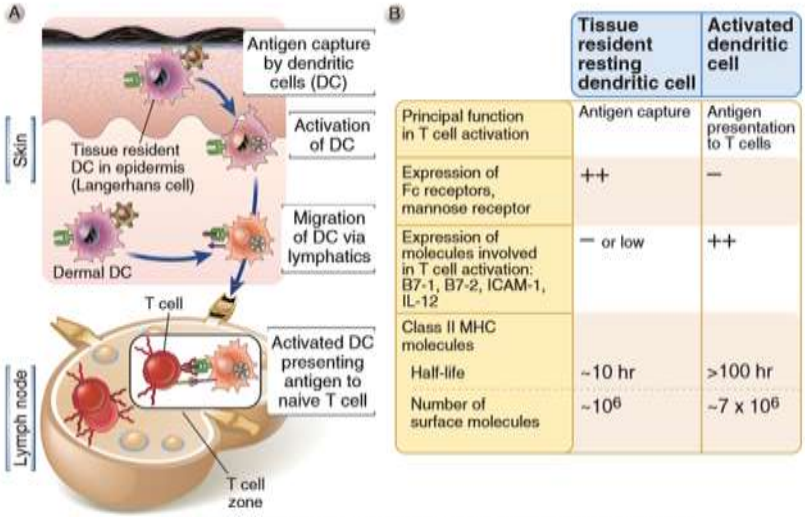

FIGURE 6.4 Role of dendritic cells in antigen capture and presentation. A, Immature DCs in the skin (Langerhans cells) or dermis (dermal DCs) capture antigens that enter through the epidermis and transport the antigens to regional lymph nodes. During this migration, the dendritic cells mature and become efficient APCs. B, The table summarizes some of the changes during dendritic cell maturation that are important in the functions of these cells.

ACTIVATED MACROPHAGES

macrophages express MHC and co-stimulatory molecules to function as antigen presenting cells after they have been activated **by cytokines such as IFN-γ,** or by having their pattern-recognition receptors (e.g., **their Toll-like receptors**) ligated by invading pathogens. Note that Macrophages don't travel

Macrophages act as "refuelling stations" which keep experienced T cells "turned on" so they can continue to participate in the battle. So mature dendritic cells activate virgin T cells, and activated macrophages mainly function to re-stimulate experienced T cells.

ACTIVATED B CELLS

The third APC is the activated B cell. A virgin B cell is not good at antigen presentation, because it expresses only low levels of class II MHC molecules and little or no B7. However, once a B cell has been activated, the levels of class II MHC molecules and B7 proteins on its surface increase dramatically. As a result, an experienced B cell is able to act as an antigen presenting cell for T helper cells.

B cells can concentrate antigen for presentation. After a B cell's receptors have bound to their cognate antigen, the whole complex of BCR plus antigen is removed from the cell surface and dragged into the cell. There the antigen is processed, loaded onto class II MHC molecules, and transported back to the cell surface for presentation.

Because B cell receptors have such a high affinity for antigen, they act like "magnets," collecting antigen for presentation to T helper(Th) cells. Since a threshold number of T cell receptors must be crosslinked by presented antigen before a Th cell can be activated, it is estimated that activated B cells have a 100- to 10 000-fold advantage over other APCs in activating helper T cells at times when there is relatively little antigen around. **Presentation of antigen by B cells is also very fast**. Less than half an hour elapses between the time antigen is captured by a B cell's receptors and the time it is displayed on the cell surface by class II MHC molecules

In summary, when an invader is first encountered, all the B cells which could recognize that particular invader are virgins, so the important APCs are activated dendritic cells. Then, while the battle is raging, activated macrophages present antigen to T cells . Later in the infection, or if this same invader is encountered again, experienced B cells are extremely important APCs – because they can quickly activate helper T cells by concentrating small amounts of antigen for presentation

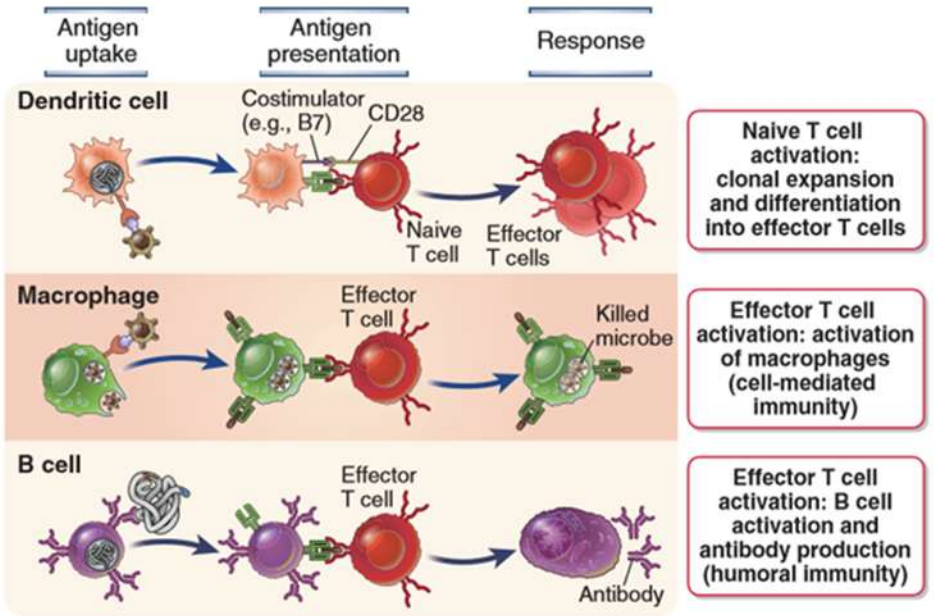

FIGURE 6.2 Functions of different antigen-presenting cells. The three major types of APCs for $CD4^+$ T cells function to display antigens at different stages and in different types of immune responses. Note that effector T cells activate macrophages and B lymphocytes by production of cytokines and by expressing surface molecules; these will be described in later chapters.

Co-Stimulation

Antigen-presenting cells that present antigens to T cells also receive signals back from these lymphocytes that enhance the antigen-presenting function of the APCs. In particular, CD4+ T cells that are activated by antigen recognition and costimulation express surface molecules, notably one called CD40 ligand (CD154), which binds to CD40 on DCs and macrophages, and the T cells also secrete cytokines, such as interferon-γ (IFN-γ), that bind to their receptors on these APCs. The **combination of CD40 signals and cytokines activates the APCs, resulting in increased ability to process and present antigens, increased expression of costimulators, and secretion of cytokines that activate the T cells. *This bidirectional interaction between APCs displaying the antigen and T lymphocytes that recognize the antigen functions as a positive feedback loop that plays an important role in maximizing the immune response***

SUMMARY OF PRESENTATION BY MHC II

-MHC II are found only on APC

-MHC II are highly polymorphic, they are encoded by HLA-D region on chromosome 6

-they are composed of alpha and beta chains which bind to another invariable protein which guards the binding site and guides MHC II in the activated APC to Golgi apparatus where it binds to the Ag (within the endosomes)

-while travelling to Golgi, the invariant chain is cleaved leaving a small chain called CLIP

-APC recognize exogenous Ag via Toll like receptors (DCs and macrophages) or BCR on B cells

-after recognition the antigen enters the cell via phagocytosis. Note in case of B cell, the BCR-Ag complex enters the cell

-the Ag is then processed by enzymatic cutting by specific enzymes that cut it to the right length to fit the MHC II binding site (groove)

-other enzymes cut CLIP protein from the MHC binding site, so the site is free to bind the processed peptide

-the MHC II attached to the peptide is expressed on the APC surface where it ca be recognized by T helper.

-the above process happens first in DC that travel to lymph nodes and stimulate naive T helpers

-stimulated T helper travels to the site of injury. During this journey macrophages continue Ag presentation to keep these T cells activated

-Activated B cells help in the process especially if the antigen was encountered before by that B cell clone.

-there is co stimulation between T cells and APCs to keep the keep the immune response going

OK; to finish the subject there are two strange ideas we need to discuss…… please remember: in medicine and especially in immunology rules always have ecxeptions.!!!!!!!!!!!

CROSS PRESEWNTATION

It has been shown that a certain subset of antigen presenting cells can take up **exogenous** antigens and shuttle them into **the class I pathway** for presentation by class I MHC molecules (instead of the usual class II) This is termed cross-presentation.

The idea is that if a clever pathogen (e.g., a virus) figured out a way to avoid infecting antigen presenting cells, yet could still infect and reproduce in other cells of the body, cross-presentation would give the immune system a chance to be activated.

NON-CLASSICAL MHC MOLECULES AND LIPID PRESENTATION

Class I and class II MHC molecules are called classical MHC molecules. There also are **non-classical** MHC molecules. The best studied of these is the **CD1 family of proteins**. These non-classical MHC molecules resemble class I MHC molecules in that they consist of a long, heavy chain protein which is paired with the β2-microglobulin protein. CD1, non-classical MHC molecules have grooves which are designed **to bind lipids**. CD1 molecules can "sample" lipids from various compartments within a cell, and can present these molecules on the surface of antigen presenting cells, where they can activate T cells. Obviously, CD1 presentation of lipids to T cells is an exception to the rule that T cells recognize peptides only. So far, however, it is not clear how important lipid presentation is for the immune defence. Consequently, I, let's "stick to the rule" that T cells only recognize protein antigens. **Be aware, however, that this may change as more research is done on CD1presented lipids.**