**Lymphocytes – activation, APC.**

Immunology 7

Ativation of lymphocytes
APC
Effector function of lymphocytes

## Characterisation of adaptive immunity

- after 1st exposition to antigen the system reacts slowly and with delay
- even if lymphocytes were screened for self reactiong TCR, they test antigens again to prevent false reactions
- to test and balance reactions is allowed by different cells that – recognise, regulate a effect reactions
- Cooperation with innate mechanisme that prepares antigens for adaptive immunity., adaptive immunity influences innate immunity reactions

## IMMUNITY: CONTRASTS BETWEEN NON-SPECIFIC AND SPECIFIC

### Non-specific (natural, native, innate)

- System in place prior to exposure to antigen
- Lacks discrimination among antigens
- Can be enhanced after exposure to antigen through effects of cytokines

### Specific (acquired, adaptive)

- Induced by antigen
- Enhanced by antigen
- Shows fine discrimination

The hallmarks of the specific immune system are memory and specificity.

- The specific immune system "remembers" each encounter with a microbe or foreign antigen, so that subsequent encounters stimulate increasingly effective defense mechanisms.
- The specific immune response amplifies the protective mechanisms of non-specific immunity, directs or focuses these mechanisms to the site of antigen entry, and thus makes them better able to eliminate foreign antigens.

All cells of the immune system originate from a hematopoietic stem cell in the bone marrow, which gives rise to two major lineages, a myeloid progenitor cell and a lymphoid progenitor cell . These two progenitors give rise to the myeloid cells (monocytes, macrophages, dendritic cells, meagakaryocytes and granulocytes) and lymphoid cells (T cells, B cells and natural killer (NK) cells), respectively. Theses cells make up the cellular components of the innate (non-specific) and adaptive (specific) immune systems.

### Cells of the innate immune system

Cells of the innate immune system include phagocytic cells (monocyte/macrophages and PMNs), NK cells, basophils, mast cells, eosinophiles and platelets. The roles of these cells have been discussed previously (see non-specific immunity). The receptors of these cells are pattern recognition receptors (PRRs) that recognize broad molecular patterns found on pathogens (pathogen associated molecular patterns, PAMPS).

## Cells that link the innate and adaptive immune systems

A specialized subset of cells called antigen presenting cells (APCs) are a heterogenous population of leukocytes that play an important role in innate immunity and also act as a link to the adaptive immune system by participating in the activation of helper T cells (Th cells). These cells include dendritic cells and macrophages. A characteristic feature of APCs is the expression of a cell surface molecule encoded by genes in the major histocompatibility complex, referred to as class II MHC molecules. B lymphocytes also express class II MHC molecules and they also function as APCs, although they are not considered as part of the innate immune system. In addition, certain other cells ( *e.g.*, thymic epithelial cells) can express class II MHC molecules and can function as APCs.

## Cells of the adaptive immune system

Cells that make up the adaptive (specific) immune system include the B and T lymphocytes. After exposure to antigen, B cells differentiate into plasma cells whose primary function is the production of antibodies. Similarly, T cells can differentiate into either T cytotoxic (Tc) or T helper (Th) cells of which there are two types Th1 and Th2 cells.

There are a number of cell surface markers that are used in clinical laboratories to distinguish B cells, T cells and their subpopulations.

Antigen processing and presentation

- Phagocyting cells screen (patrol) environment – phagocytosis, macropinocytosis
- ensymatically split engulfed proteins - degradation
- some fragments of proteins are caught and presented by MHC II – **presentation of antigens**
- some microbes are not phagocyted, or degraded and are processed and presented by MHC I molecules

## ANTIGEN PROCESSING AND PRESENTATION

Antigen processing and presentation are processes that occur within a cell that result in fragmentation (proteolysis) of proteins, association of the fragments with MHC molecules, and expression of the peptide-MHC molecules at the cell surface where they can be recognized by the T cell receptor on a T cell. However, the path leading to the association of protein fragments with MHC molecules differs for class I and class II MHC. MHC class I molecules present degradation products derived from intracellular (endogenous) proteins in the cytosol. MHC class II molecules present fragments derived from extracellular (exogenous) proteins that are located in an intracellular compartment.

### Antigen processing and presentation in cells expressing class I MHC

All nucleated cells express class I MHC. Proteins are fragmented in the cytosol by proteosomes (a complex of proteins having proteolytic activity) or by other proteases. The fragments are then transported across the membrane of the endoplasmic reticulum by transporter proteins. (The transporter proteins and some components of the proteosome have their genes in the MHC complex). Synthesis and assembly of class I heavy chain and beta$_2$ microglobulin occurs in the endoplasmic

reticulum. Within the endoplasmic reticulum, the MHC class I heavy chain, beta$_2$microglobulin and peptide form a stable complex that is transported to the cell surface.

## ANTIGEN PRESENTING CELLS

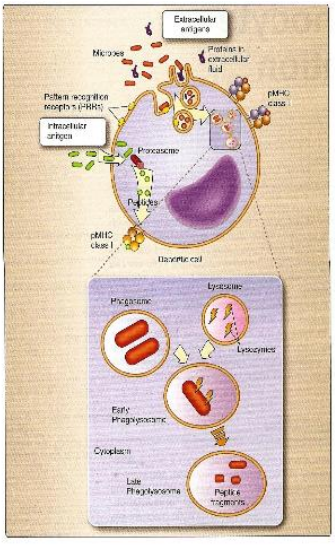
The three main types of antigen presenting cells are dendritic cells, macrophages and B cells, although other cells, that express class II MHC molecules, (*e.g.*, thymic epithelial cells) can act as antigen presenting cells in some cases. Dendritic cells, which are found in skin and other tissues, ingest antigens by pinocytosis and transport antigens to the lymph nodes and spleen. In the lymph nodes and spleen they are found predominantly in the T cells areas. Dendritic cells are the most effective antigen presenting cells and can present antigens to naïve (virgin) T cells. Furthermore, they can present internalized antigens in association with either class I or class II MHC molecules (cross presentation), although the predominant pathway for internalized antigen is the class II pathway. The second type of antigen presenting cell is the macrophage. These cells ingest antigen by phagocytosis or pinocytosis. Macrophages are not as effective in presenting antigen to naïve T cells but they are very good in activating memory T cells. The third type of antigen presenting cell is the B cell. These cells bind antigen via their surface immunoglobulin and ingest antigens by pinocytosis. Like macrophages these cells are not as effective as dendritic cells in presenting antigen to naïve T cells. B cells are very effective in presenting antigen to memory T cells, especially when the antigen concentration is low because surface immunoglobulin on the B cells binds antigen with a high affinity.

### Antigen processing and presentation in cells expressing class II MHC

Whereas all nucleated cells express class I MHC, only a limited group of cells express class II MHC, which includes the antigen presenting cells (APC). The principal APC are macrophages, dendritic cells (Langerhans cells), and B cells, and the expression of class II MHC molecules is either constitutive or inducible, especially by interferon-gamma in the case of macrophages.

Exogenous proteins taken in by endocytosis are fragmented by proteases in an endosome. The alpha and beta chains of MHC class II, along with an invariant chain, are synthesized, assembled in the endoplasmic reticulum, and transported through the Golgi and trans-Golgi apparatus to reach the endosome, where the invariant chain is digested, and the peptide fragments from the exogenous protein are able to associate with the class II MHC molecules, which are finally transported to the cell surface.

- i.c.pathogens
- degraded
- MHC I




extracelular
processed
MHC II

## Intracellular pathogens

Because antibodies do not get into host cells, they are ineffective against intracellular pathogens. The immune system uses a different approach to deal with these kinds of pathogens. Cell-mediated responses are the primary defense against intracellular pathogens and the approach is different depending upon where the pathogen resides in the host cell (*i.e.,* in the cytosol or within vesicles). For example, most viruses and some bacteria reside in the cytoplasm of the host cell, however, some bacteria and parasites actually live within endosomes in the infected host cell. The primary defense against pathogens in the cytosol is the cytotoxic T lymphocyte (Tc or CTL). In contrast, the primary defense against a pathogen within vesicles is a subset of helper T lymphocytes (Th1).

- **Cytotoxic T lymphocytes**
  CTLs are a subset of T lymphocytes that express a unique antigen on their surface called CD8. These cells recognize antigens from the pathogen that are displayed on the surface of the infected cell and kill the cell thereby preventing the spread of the infection to neighboring cells. CTLs kill by inducing apoptosis in the infected cell.
- **Th1 Helper T cells**
  Th cells are a subset of T cells that express a unique antigen on their surface called CD4. A subpopulation of Th cells, Th1 cells, is the primary defense against intracellular pathogens that live within vesicles. Th1 cells recognize antigen from the pathogen that are expressed on the surface of infected cells and release cytokines that activate the infected cell. Once activated, the infected cell can then kill the pathogen. For example, *Mycobacterium tuberculosis*, the causative agent of tuberculosis, infects macrophages but is not killed because it blocks the fusion of lysosomes with the endosomes in which it resides. Th1 cells that recognize *M. tuberculosis* antigens on the surface of an infected macrophage can secrete cytokines that activate macrophages. Once activated the lysosomes fuse with endosomes and the *M. tuberculosis* bacteria are killed.

Although immune responses are tailored to the pathogen and to where the pathogen resides, most pathogens can elicit both an antibody and a cell-mediated response, both of which may contribute to ridding the host of the pathogen. However, for any particular pathogen an antibody or a cell-mediated response may be more important for defense against the pathogen.

## Extracellular pathogens

Antibodies are the primary defense against extracellular pathogens and they function in three major ways:

- **Neutralization**
  By binding to the pathogen or foreign substance antibodies, can block the association of the pathogen with their targets. For example, antibodies to bacterial toxins can prevent the binding of the toxin to host cells thereby rendering the toxin ineffective. Similarly, antibody binding to a virus or bacterial pathogen can block the attachment of the pathogen to its target cell thereby preventing infection or colonization.
- **Opsonization**
  Antibody binding to a pathogen or foreign substance can opsonize the material and facilitate its uptake and destruction by phagocytic cells. The Fc region of the antibody interacts with Fc receptors on phagocytic cells rendering the pathogen more readily phagocytosed.
- **Complement activation**
  Activation of the complement cascade by antibody can result in lysis of certain

bacteria and viruses. In addition, some components of the complement cascade (*e.g.* C3b) opsonize pathogens and facilitate their uptake via complement receptors on phagocytic cells.

## Presentation via MHC II

- Dendritic cells – in sites of gate of entry of antigens
- Not mature cell engulf free and bound molecules / antigens – phagocytosis and degradation
- Recognition via PRR receptors – directly (non specific) or
  indirectly via antibodies(specific)

## Recognition of threat leads to maturation of dendritic cells

- migration closly to lymphatic nodes
- decrease phagocyting activity
- increase synthesis of MHC II
- transport of MHC II with bound peptid = pMHC II to the surface of dendritic cell and presentation of antigen to CD4

## Presentation via MHC I

- Not all antigens enter the cell by phagocytosis.
- Some are bound on the surface of target cells that infects
- The cell degrades them by proteasomes and binds them to MHC I – to produce pMHC I, that is exposed and cooperates with CD8

## Activation of T lymphocytes a) immunological synapsis

- T lymphocytes – direct reactions of adaptive immunity
- TCR does not recognise free molecules
- recognise only peptids presented via MHC I or MHC II
- Specific immunity is influenced by the way how the epitopes are presented by Antigen Presenting Cells

## Immunological synapsis:

- TCR recognises pMHC
- pMHC + TCR of CD4 or CD8 – stabilisation is done by constant part of TCR =
  **TCR:pMHC:CD4(8)** = 1st signal via CD3 to Tcell
- 2nd signal – **costimulating molecules**
- 1st and 2nd signal = transmission of signal and i.c. activation of transcription of genes for production of cytokines
- Without costimulation the lymphocytes will be selectively non-respondint **(anergy)** or will be submitted for apapotosis

## CD4 maturation

- T cell + antigen = **priming.**
- Primed CD4 **= T helper**
- **Thp** are precursors of Th **=** stimulated and secretes cytokines
- **Th0 –** developes further by one of 2 functional ways accordinig to the character of contact with APC
- **Th1:** microbial LPS + cytokine from APC( IL12) = activation of phagocytosis and CTL
- **Th2** - IL 4 leads to Th0 development to Th2 that stimulates B ly to change to plasmatic cells and memory cells

## CENTRAL ROLE OF TH CELLS IN IMMUNE RESPONSES

After Th cells recognize specific antigen presented by an antigen-presenting cell (APC), they can initiate several key immune processes. These include:

- Selection of appropriate effector mechanisms ( e.g., B cell activation or Tc generation);
- Induction of proliferation of appropriate effector cells
- Enhancement of the functional activities of other cells (e.g., granulocytes, macrophages, NK cells).

There are four subpopulations of Th cells: Th0, Th1, Th2 and Th17 cells. When naïve Th0 cells encounter antigen in secondary lymphoid tissues, they are capable of differentiating into inflammatory Th1 cells, helper Th2 cells or pathogenic T17 cells, which are distinguished by the cytokines they produce. Whether a Th0 cells becomes a Th1, a Th2 or a T17 cell depends upon the cytokines in the environment, which is influenced by antigen. For example some antigens stimulate IL-4 production which favors the generation of Th2 cells while other antigens stimulate IL-12 production, which favors the generation of Th1 cells. Th1, Th2 and Th17 cells affect different cells and influence the type of an immune response, as shown in Figure 3 for Th1 and Th2 cells.

Cytokines produced by Th1 cells activate macrophages and participate in the generation of cytoxic lymphocytes (CTL), resulting in a cell-mediated immune response. In contrast cytokines produced by Th2 cells help to activate B cells, resulting in antibody production.

In a relatively recent discovery, Th17 cells (designated as such by their production of IL-17) differentiate (in humans) in response to IL-1, IL-6, and IL-23. TGF-β is important for Th17 differentiation in mice, but not in humans. IL-17 enhances the severity of some autoimmune diseases including multiple sclerosis, inflammatory bowel disease, and rheumatoid arthritis. Equally important, each subpopulation can exert inhibitory influences on the other. IFN-γ produced by Th1 cells inhibits proliferation of Th2 cells and differentiation of Th17 cells and IL-10 produced by Th2 cells inhibits production of IFN-γ by Th1 cells. In addition, although not shown, IL-4 inhibits production of Th1 cells and differentiation of Th17 cells. Thus, the immune response is directed to the type of response that is required to deal with the pathogen encountered – cell-mediated responses for intracellular pathogens or antibody responses for extracellular pathogens.

## CD8 - maturation

- Recognition of pMHC I on the surface of (APC, infected or other) cells by CD8 – formation of **IL-2 receptor**
- IL 2 is produced by CD4, that communicated with the same antigen in MHC II on APC (phagocyted fragments of microbe body)
- IL 2 **stimulates CD8** to differenciate to CTL – cytotoxic T cells – containing cytolytical granules (perforins and granzymes), that kill cell with specific pMHC I

## CELL-CELL INTERACTIONS IN CELL-MEDIATED IMMUNITY - GENERATION OF TC CELLS IN RESPONSE TO ENDOGENOUS ANTIGENS IN THE CYTOSOL

Cytotoxic T lymphocytes are not fully mature when they exit the thymus. They have a functional TCR that recognizes antigen, but they cannot lyse a target cell. They must differentiate into fully functional effector Tc cells. Cytotoxic cells differentiate from a "pre-CTL" in response to two signals:

- Specific antigen in the context of class I MHC, on a stimulator cell
- Cytokines produced by Th1 cells, especially IL-2, and IFN-gamma.

## Features of CTL-mediated lysis

- CTL killing is antigen-specific. To be killed by a CTL, the target cell must bear the same class I MHC-associated antigen that triggered pre-CTL differentiation.
- CTL killing requires cell contact. CTL are triggered to kill when they recognize the target antigen associated with a cell surface MHC molecule. Adjacent cells lacking the appropriate target MHC-antigen are not affected.
- CTLs are not injured when they lyse target cells. Each CTL is capable of killing sequentially numerous target cells.

## Mechanisms of CTL-mediated killing

CTLs utilize several mechanisms to kill target cells, some of which require direct cell-cell contact and others that result from the production of certain cytokines. In all cases death of the target cells is a result of apoptosis.

- Fas- and TNF-mediated killing
  Once generated CTLs express Fas ligand on their surface, which binds to Fas receptors on target cells. In addition, TNF-α secreted by CTLs can bind to TNF receptors on target cells. The Fas and TNF receptors are a closely related family of receptors, which when they encounter their ligands, for trimers of the receptors. These receptors also contain death domains in the cytoplasmic portion of the receptor, which after trimerization can activate caspases that induce apoptosis in the target cell.

- Granule-mediated killing
  Fully differentiated CTLs have numerous granules that contain perforin and granzymes. Upon contact with target cells, perforin is released and it polymerizes to form channels in the target cell membrane. Granzymes, which are serine proteases, enter the target cell through the channels and activate caspases and nucleases in the target cell resulting in apoptosis.

## Memory cells

- TCR + pMHC II (**first signal**)
- CD 28 + APC (CD80/86) **2nd signal** that leads to
  - production of IL 2 by CD4
  - to formation of IL 2 receptor on CD8 and
  - to proliferation (negative stimulation – selfregulation to stop if prolifer too much )
- Some CD4 become memory cells – have more CD 28 – react much faster to APC and migrate to the place of infection (do not stay at LNodes)

## Activation of B cells

- BCR recognise and process free and bound antigens
- BCR has the shape of monomer of IgD and/or IgM
- Cytoplasmatic part of Ig – play the role of CD3 of Tcells
- Binding of epitop on Ig leads to transmission of signal and to transsduction and transcription and activation of genes, endocytosis, ensymatic degradation amd exposition of fragments by pMHC II and to production of costimulationg molecules
- B cells can act as APC

## T independent antigens
## T independent activation

TI activates B lymphocytes without T cell

- TI-1 antigens – polyclonal activators of proliferation and Ig production = **mitogens of B cells**
- T1-2 antigens – contain repeated epitopes (polysacharides), activate mature B cells (transmission of signals) – in minimal quantity activates also T cells

2nd signal – other bindings of B cell – coreceptors on B cells (C3b, CD 21= CR2, CR1...)

## CELL-CELL INTERACTIONS IN ANTIBODY RESPONSES TO EXOGENOUS T-INDEPENDENT ANTIGENS

Antibody responses to T-independent antigens do not require cell-cell interactions. The polymeric nature of these antigens allows cross-linking of antigen receptors on B cells resulting in activation. No secondary responses, affinity maturation or class switching occurs. Responses to T-independent antigens are due to the activation of a subpopulation of B cells called CD5+ B cells (also called B1 cells), which distinguishes them from conventional B cells that are CD5- (also called B2 cells).

### CD5+ (B1) cells

CD5+ cells are the first B cells to appear in ontogeny. They express surface IgM but little or no IgD and they produce primarily IgM antibodies from minimally somatically mutated germ line genes. Antibodies produced by these cells are of low affinity and are often polyreactive (bind multiple antigens). Most of the IgM in serum is derived from CD5+ B cells. CD5+ B cells do not give rise to memory cells. An important characteristic of these cells is that they are self-renewing, unlike conventional B cells which must be replaced from the bone marrow. CD5+ B cells are found in peripheral tissues and are the predominant B cell in the peritoneal cavity. B1 cells are a major defense against many bacterial pathogens that characteristically have polysaccharides in their cell walls. The importance of these cells in immunity is illustrated by the fact that many individuals with T cell defects are still able to resist many bacterial pathogens.

## T dependent antigens and activation

- Antigens bound via MHC II (on APC or B cells) – first signal
- 2nd signal to B lymphocyte is given by CD4 helper cell
- MHC II + TCR CD4 cells = production of cytokines (IL4) and formation of IL4 receptore on B cells – proliferation of B cells, differenciation to plasmatic cell

SOURCE: IMMUNOLOGY - CHAPTER TWELVE CELL-MEDIATED IMMUNITY: Cell-cell interactions in specific immune responses

Available from: http://www.microbiologybook.org/bowers/cell-mediated-ver2.htm