Cell-Mediated Immunity:

Introduction;

Fig 12.1 Khan 2009

- Cell-mediated immunity refers to those immune reactions that occur on or near the cells and
- In which immune cells are directly involved
- Cell mediated immune response can be antigen-specific as well as non-specific
- The non-specific arm of cell-mediated immunity includes cells such as
  - Macrophages
  - Neutrophils
  - Eosinophil and
  - Natural killer (NK) cells
- These cells bear pattern-recognition receptors such as
  - Toll-like receptors (TLR) and
  - Scavenger receptor
Figure 12.1
Antibody-dependent cell-mediated cytotoxicity by NK cells.
That recognize conserved structures present on the surface of pathogens called pathogen associated molecular patterns (PAMP).

PAMPs includes:
- Bacterial lipopolysaccharide
- Peptidoglycan
- Double stranded RNA etc.
- NK cells also contribute to non-specific defense by binding non-specifically to antibody-coated cells and inducing target cell lysis.

Fig 12.2. Khan 2009

Recently it has been suggested that some other molecules i.e. Nod proteins (Nod 1 and Nod 2) are also involved in non-specific immunity.

These proteins are located intracellularly and represent an important defense against invasive Gram-negative pathogens such as:
- *Shigella flexineri*
- *E.coli*
- *Streptococcus pneumoniae* and
Figure 12.2
Schematic diagram showing the non-specific arm of cell-mediated immunity. (PAMP—pathogen-associated molecular patterns, such as lipopolysaccharide, lipoteichoic acid and mannan; PRR—pattern-recognition receptors, such as Toll-like receptor, scavenger receptor; MBP—major basic protein.)
Pseudomonas aeruginosa

• Nod 1 is reported to occur in epithelial cells
• The specific cells include $T_{cyt}$ and cytokine-secreting $T_H$ cells
• Both cells need to interact directly with the cells they are going to kill or help, depending on the cell type and
• They do this through a specific recognition mechanism
• Antigen-specific $T_{cyt}$ cells perform the effector function by directly lysing the target cells
• $T_{cyt}$ and $T_H$ cells are activated specifically by a particular antigen plus MHC and may cause cell death by activating the target cell or
• May activate and recruit non-specific effector cells, such as macrophages, neutrophils, eosinophils and natural killer cells

Fig 12.3 Khan 2009

• The target cells that are killed by non-specific effector cells use bound antibody to target cell as a signal for killing
• The activity of both specific and non-specific is regulated by a concentration of a variety of different cytokines
A small percentage of CD4+ T cells also show cytolytic activity. This cytolytic activity is mediated through the Fas pathway that kills the target cell without the use of perforin (used by Tc1). In vitro studies have shown that these cells form a very effective defence against certain viruses such as the Epstein-Barr virus.
- **Lymphocytes and Cell-Mediated Response:**

  - Cell mediated immunity refers to immunity rendered by cells of the immune system.

  - This form of immunity can be transferred to a non-immune individual by the transfer of cells and not by the serum or plasma.

  - There are two major subsets of effector cells that are involved in cell-mediated response.
    - One subset comprises specific effector cell such as T<sub>c</sub>yt cells and non-specific effector cells such as NK cells and macrophages that are involved in the lysis of target cells.
    - The other subset comprises CD4+ T<sub>H</sub> cells that mediate delayed-type hypersensitivity reactions.

  **Table 12.1 Khan 2009**

- **Cytotoxic T Lymphocytes and Mechanism of Cytotoxicity**

  - Cytotoxic T lymphocytes (T<sub>c</sub>yt) are subset of T cells that express CD8 antigens together with TCR on their surface.

  **Fig 12.4 Khan 2009**
### Table 12.1

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Effector Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>T$_{eff}$ cell</td>
<td>Perforin, granzyme, Fas ligand, TNF-β, IFN-γ</td>
</tr>
<tr>
<td>NK cell</td>
<td>Perforin, granzyme, IFN-γ, TNF-α, TNF-β, FcγRIII that mediates ADCC</td>
</tr>
<tr>
<td>T$_{reg}$ cell</td>
<td>IL-2, TNF-β, IFN-γ</td>
</tr>
<tr>
<td>T$_{H1}$ cell</td>
<td>IL-3, IL-4, IL-5, IL-10, IL-13, CD40 ligand.</td>
</tr>
<tr>
<td>Macrophage</td>
<td>IL-1, IL-6, IL-12, IFN-γ, TNF-α, GM-CSF, enzymes, prostaglandins, complement proteins</td>
</tr>
</tbody>
</table>

*Note: ADCC—Antibody-dependent cell-mediated cytotoxicity; IFN interferon; TNF tumour necrosis factor; IL—interleukin.*
Figure 12.4
Schematic diagram showing generation of mature $T_{cyt}$ cells after activation by T-helper cytokines and peptide–MHC costimulatory molecules. T cells exiting the thymus are mature but innocent. The differentiation of these pre-$T_{cyt}$ cells into functional $T_{cyt}$ cells requires the presence of specific antigens on class I MHC, stimulation by IL-2 secreted by $T_{H1}$ cells, costimulation by members of the B7 family present on target cells (IL-2—interleukin 2, IL-2R—interleukin-2 receptors).
There are two pathways:

- Perforin/Granzyme pathway
- Fas pathway

Recent studies have suggested that between the perforin/granzyme pathway and the fast pathway, the perforin/granzyme pathway is the key mediator of cytotoxic function of $T_{cyt}$ cells.

However, both pathways share some common steps that involved the adhesion of target cells with $T_{cyt}$ cells.

Fig 12.5 Khan 2009
Fig 12.6 Khan 2009
Fig 12.7 Khan 2009

- Natural Killer Cells and Mechanism of Cytotoxicity

NK cells form a distinct group of lymphocytes with no immunological memory and are independent of the adaptive immune system.

Fig 12.8 Khan 2009
Fig 12.9 Khan 2009
Cytolitic Function
Cytolytic function is the ability to lyse cells. Examples of cells having cytolytic capabilities include T_{cyt} cells and NK cells.

Figure 12.5
Diagram showing interaction of T_{cyt} cells and helper T cells with ligands. The molecules on T cells are known to be involved in interactions between T cells and APC (CTL4—cytotoxic T lymphocyte 4; LFA1—lymphocyte-associated function antigen 1, IFN—interferon).
- **T<sub>cyt</sub>-target cell conjugate**
- Microtubule organization of cytoskeleton changes in such a way that perforin and granzyme granules move to contact area of the T cell with the target cell
- Fusion of granule membranes occurs with the T-cell membrane
- Resulting in exocytosis of granule contents into the extracellular region
- The fusion of granular membrane is believed to be Ca<sup>2+</sup>-dependent and thought to involve Rab - a Ras associated binding protein

* ICE-Interleukin-1 converting enzyme
** Because of osmotic swelling
This is an additional mechanism by which an apoptotic pathway can be triggered in target cell.

It was found that some CD4+ cytotoxic cells do not contain perforin-granzyme but were able to lyse the target cell in the absence of Ca2+.

The binding of FasL to Fas molecule on the target cell results in its clustering.

Fas activates another protein-FADD (Fas-associated protein with death domain) which associates with a second protein FLICE-FADD-like interleukin converting enzyme protease.
- KAR - killer activatory receptor
- KIR – Killer inhibitory receptor

**Figure 12.8**
Line diagram of NK cell showing KAR and KIR. In humans, KIR recognize and bind directly to the conserved section of the class I MHC molecules. CD94/NKG2 recognize peptides from classical class I MHC molecules displayed on non-classical class I MHC molecules. KAR recognize KAR ligand displayed on healthy cells (KIR—killer inhibitory receptor; KAR—killer activatory receptor).
**Figure 12.9**
NK cells and their toxicity and recognition.

Different ways of target cell recognition by NK cell.
Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC);

- ADCC is the killing of antibody-coated target cells by non-phagocytic mechanism in which effector cells bind the Fc region of the antibody and lyse the target cell.

- Effector cells involved in this form of cell mediated cytotoxicity are:
  - NK cells
  - Macrophages
  - Neutrophils
  - Monocytes and
  - Eosinophils

- The antibody, usually IgG or IgE, IgA, specific for a structure on a target cell membrane, coats the target cell.

- The Fc receptor-bearing effector cells then bind to antibodies already attached to antigen on a target cell and

- Subsequently cause target cell death.
Figure 12.10
Mechanism of NK-cell toxicity. The roles of only KIR and KAR are shown.
• These “cytotoxic” cells are non-specific for antigen since they bind to the Fc region of IgG specifically IgG1 or IgG3, specificity of the antibody directs to specific target cell

• This is known as **antibody-dependent cell-mediated cytotoxicity (ADCC)**

  **Fig 12.11 Khan 2011**

### Delayed Type Hypersensitivity (DTH)

• Tuberculosis is one of the leading scourges of 19\textsuperscript{th} century industrialized Western world

• Robert Koch identified its causative organism as tubercle bacillus in 1882

• He and his colleague Louis Pasteur looked forward to conquer this disease by vaccinating as well as treating infected individual by tuberculin, a product of the tubercle bacilli culture

• The intravenous injection of tuberculin in tuberculosis patients let to server systemic reaction and occasionally death

• Accompanying all these disappointing results was one ray of hope

• He discovered in 1891 that if tuberculin was applied intradermally, local inflammatory reaction would be elicited in those individuals that had this disease
Figure 12.11
Different cytotoxic mechanisms of antibody-dependent cell-mediated cytotoxicity.
• This was termed as delayed-type skin test as it took 24-48 hours to develop or specifically, tuberculin reaction

• This tuberculin reaction helped to isolate and detect patients having tuberculosis during those days

• With the discovery that similar reaction could be elicited by luetin (extract of treponemes for syphilis), lepromin (extract from Hansen’s bacillus for leprosy) as well as other bacterial extract, these reactions termed as delayed skin reactions and later delayed-type hypersensitivity

• Finally in 1942, Landsteiner and Chase demonstrated for the first time that contact hypersensitivity to picryl chloride could be transferred from one guinea pig to another naïve recipient via live peritoneal cells

• The recipient guinea pig was found to elicit positive skin test to picryl chloride 24 hours later

• No transfer of delayed hypersensitivity could be obtained using the fluid phase from the exudates or killed cells

• These experiments proved that delayed type hypersensitivity reaction is mediated by cells not by circulatory antibodies dissolve in the fluid phase

• DTH is a form of cell-mediated immune response that involves TH cells and non-specific effector cells such as activated macrophages
• Since the ultimate effector cells are non-specific and since they can not differentiate between self and invading pathogen, some degree of host damage is done

• Symptoms appear after some delay following antigen exposure i.e. usually 24-72 hours

• Hence it is called as delayed-type hypersensitivity

• The term hypersensitivity is attached here because DTH reactions sometimes produce tissue injury without providing any protective function

• However, it should not be assumed that DTH reactions are always harmful

• In fact, this type of cell-mediated immunity is a part of primary defense mechanism against intracellular bacteria such as:

  ✓ *Mycobacterium leprae*
  ✓ *Mycobacterium tuberculosis*
  ✓ *Listeria monocytogenes*

• When pathogen enter the host body, they are phagocytosed by cells of innate immunity such as macrophages

• However, some pathogens such as *Mycobacterium tuberculosis* can actually survive inside the phagocyte’s cytoplasm or lysosome
The killing of such intracellular bacteria requires switching on previously subverted microbial mechanism in the phagocyte by $T_H$ cell-derived cytokines

- **Sensitization and Effector Phases in DTH Response**
  - DTH reactions are produced in two phases;
    - The sensitization or activation phase
    - The effector phase

  ![Fig 12.12 Khan 2009](image)
  ![Fig 12.13 Khan 2009](image)

- **Cytokines and DTH Reaction**
  - A large variety of cytokines play a important role in shaping DTH reaction
  - In DTH reactions, cytokines are secreted by
    - $T_{DTH}$ cell
    - Macrophages and sometimes even by
    - Keratinocytes

  ![Fig 12.14 Khan 2009](image)
Figure 12.12
Line diagram showing sensitization phase of DTH reaction. $T_{H1}$ cells comprise mainly $CD^4^+T$ cells, but may also include $CD^8^+T$ cells.
Effector response to DTH is initiated after secondary exposure of antigen to T\textsubscript{DTH} cells.

After the second encounter with the antigen, the T\textsubscript{DTH} cells secrete cytokines that recruit macrophages and other non-specific cells at the inflamed site where antigen has entered or is localized.

Adhesion molecules bind blood monocytes causing their rolling along the endothelial surface in the direction of blood flow.

This rolling activates the monocytes which then transmigrate through inter-endothelial cell junctions of the capillaries into the neighboring tissue.

Where they differentiated into activated macrophages.

Macrophages are major effector cells of DTH response.
IL-2 functions in an autocrine and paracrine manner to amplify the response act on vascular endothelial cells to induce an activated state.

Activated macrophages increases expression of (a) Class II MHC (b) B7 co-stimulator molecules and secretes several cytokines.

Expression of adhesion molecules on endothelial cells.

These adhesion molecules cause leukocyte binding, rolling and recruitment to the site of the reaction.
<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Sources</th>
<th>Major Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>T&lt;sub&gt;H&lt;/sub&gt; cells, NK cells</td>
<td>Enhanced activity of B, T cells, NK cells</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>T&lt;sub&gt;H&lt;/sub&gt; cells, NK cells</td>
<td>Enhanced antimicrobial activity of macrophage, NK cells, depresses viral growth</td>
</tr>
<tr>
<td>TNF-α, TNF-β</td>
<td>T&lt;sub&gt;H&lt;/sub&gt; cells, macrophages</td>
<td>Induce apoptosis in tumour cells, enhances activity of B-cell, neutrophils.</td>
</tr>
<tr>
<td>IL-12</td>
<td>Macrophages, dendritic cells</td>
<td>Induce differentiation and proliferation of T&lt;sub&gt;TF&lt;/sub&gt; cells, T&lt;sub&gt;H&lt;/sub&gt; cells</td>
</tr>
<tr>
<td>Chemokines</td>
<td>Macrophages, endothelial cells, T-cells</td>
<td>Lymphocyte recruitment, active in inducing inflammation</td>
</tr>
</tbody>
</table>

Table 12.2 Cytokines involved in DTH reactions
Detection of DTH Reaction

- The presence of DTH reaction or $T_{DTH}$ cells in an individual can be experimentally observed.

- For example, to determine whether individuals have T cell-mediated immunity against tuberculosis, Mantoux test is performed developed in 1907 by French Physician.

- In this test a small amount of the purified protein derivative (PPD) obtained from cell wall of *Mycobacterium tuberculosis* is injected into skin of the individual and site is examined between 48-72 hours.

- A positive skin test shows up as firm red swelling, maximal at 48-72 hours after injection.

- This is a clinical evidence of tuberculosis infection in the individual and presence of $T_{DTH}$ cells.

- This does not allow differentiation between those individuals that carry sensitized $T_{DTH}$ cells for tuberculosis because of exposure to the pathogen and those that have sensitized because of the vaccination.

Significance of DTH Reaction

- It has two kinds of roles:
Protective

- The DHT response is one of the types of cell-mediated effector mechanism employed by our body against a variety of intracellular pathogens and contact antigens.

- In contrast to very specific T<sub>cyt</sub> responses, the DTH response non-specifically destroys cells that harbor intracellular pathogens.

- Mechanism we have already discussed.

- However, the enzymes which released to take care are unable to differentiate between cells that harbor pathogen and normal cells, a small amount of healthy tissue is also damaged.

- However, this is a small price to pay for elimination of intracellular pathogen.

Pathological Consequences

- Sometimes in DTH reaction, the source of the antigen is not completely eradicated or degraded.

- This, in turn, leads to the aggregation and proliferation of macrophages stimulated by T<sub>H</sub> cells.
- Continual stimulation of macrophages and other non-specific effector release lytic lysosomal enzymes meant to destroy persistence chronic infection

- These enzymes cause extensive damage and

- Hence at that particular moment its damaging pathologic response of DTH far outweighs and any beneficial effects
The End
Good Luck
and
Have Great Success in Examination