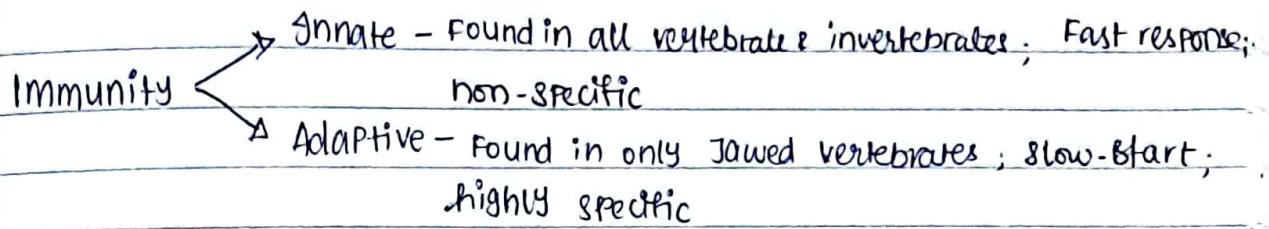


INNATE IMMUNITY

- 99% infections are blocked by anatomical barriers.
- First cell type to encounter infection inside the body is macrophages.
- If the pathogens still persist in the system, then the effector cells (mobile macrophages that is circulated through the body) come to site of infection.
- PAMP (Pathogen Associated molecular Patterns) that are present uniquely on the pathogens.
- PRR (Pattern Recognition Receptors) will recognise PAMPs present on pathogens.
- Binding will result in certain gene expressions that will express cytokines and Acute Phase Proteins.
- These proteins will induce conditions of inflammation (digestion of blood vessels → increase in diameter of blood vessels → increased blood flow → increased recruitment of immune cells)
- During the process of neutralisation of pathogens (Phagocytosis), the Antigenic Peptides are presented on the macrophage or dendritic cells.
- These APCs as well as the free antigens will travel to the secondary lymphoid organs (mainly lymph nodes).
- The APC will interact with naive B & T - cells & activate them in the sec. lymphoid organs.
- The B/T cells will undergo Clonal Expansion (multiplication, proliferation & differentiation of specific clones specific for that antigen).
- Th cells will activate B cells to differentiate into Plasma cells.
- After eradicating infection Tc, Th & B cells undergo apoptosis while only T_M memory cells will last.



INNATE IMMUNE SYSTEM:

- (1) ^{1st line of defense} Physical / Anatomical Barriers — skin, mucous membrane
- (2) Chemical Barriers — stomach acidity, antimicrobial molecules (tears)
- (3) Cellular Barriers — cells with receptors that can detect microbes (such as macrophages, dendritic cells).

Pathogens → intracellular → viral infections
 → extracellular → most bacterial infections

recognised by
Tissue-resident macrophages. — secretes cytokines & chemokines.
 via PRRs. recruitment of dendritic cells. — secretes anti-microbial peptides.
 Dendritic cells activate Adaptive immune system
 in the 2° lymphoid organs.

NOTE: Tumour cells (may not be pathogen) ^{considered} are considered intracellular, similar in action to that of virally infected cells.

08/03/22 Innate immunity:

- (i) skin
 - Epithelial cells secrete anti-microbial enzymes
 - First line of defense
 - secretes fatty acids in sebum

- (ii) mouth & alimentary canal
 - Anti-microbial enzymes
 - Hydrolytic enzymes

(iii) Stomach

→ Low pH

→ Digestive enzymes, antimicrobial Peptides

→ Fluid flow to intestine

(iv) Small intestine

→ Digestive enzymes, antimicrobial Peptides

→ Fluid flow to large intestine

Fluid flow → unidirectional;

(v) Large intestine

helps prevent backward

(vi) Airway and lungs

infection (of cells preceding

→ Cilia sweep mucus outward

them).

(vii) Urogenital tract

(viii) Salivary, Lacrimal, & mammal glands

Antimicrobial Proteins :

→ Lysozymes - breaks down cell wall

- Present in tears, saliva, respiratory tract

→ Lactoferrin - Binds & sequesters iron hindering pathogen growth

- Present in milk, intestine mucus.

→ Calprotectin - It also binds & sequesters divalent cations.

- Secreted by skin, glandular secretions

→ Secretory Leukocyte Protease inhibitor - Inhibits Proteases that imparts Pathogenicity to the microbes.

→ Defensins & Cathelicidin - Anti-microbial Peptides that are Lysine rich and are

- Cationic in nature.

- They bind to -vely charged phospholipids
Present on Pathogen cell membrane.

→ Surfactant Proteins - works as a surfactant disrupting Pathogen's membranes

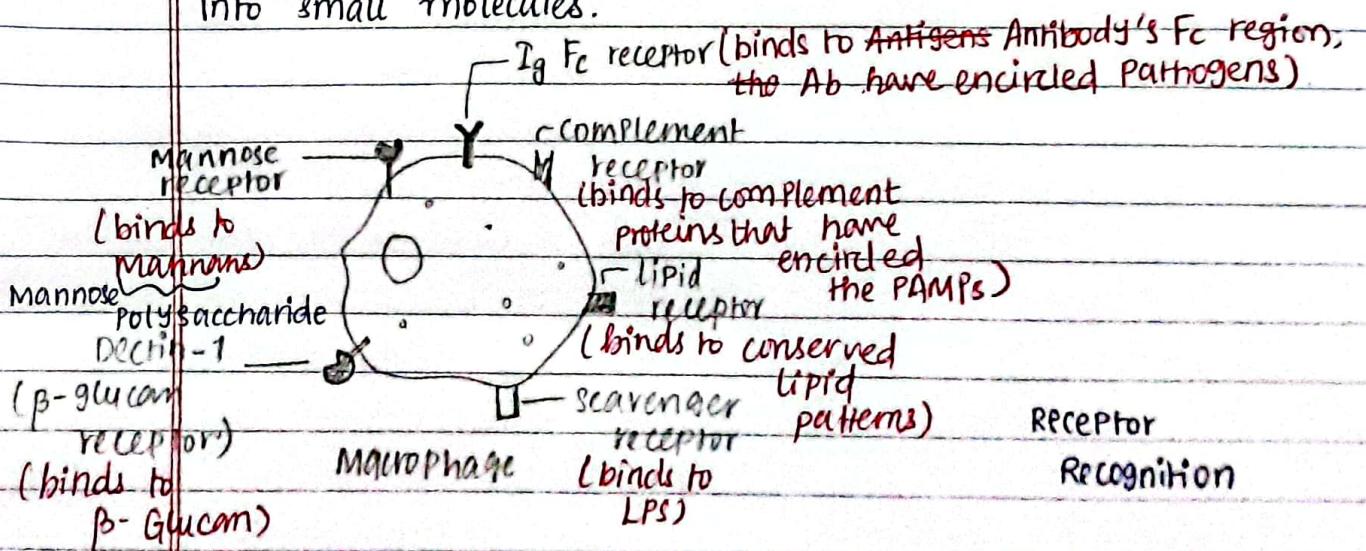
PHAGOCYTOSIS:

Most common — 1st line of Defense inside the body
Tissue-resident macrophages, Dendritic cells, Neutrophils — can
Phagocytize Pathogens.

{ Extra-cellular }
 |
 { Pathogens }

MODE OF ACTION:

- (i) Recognition of Antigen with the help of PRRs (that helps recognise conserved patterns on the Antigen — PAMPs)
- (ii) Activation of Signal transduction Pathway
- (iii) Extension of membrane of Phagocyte area (Pseudopodia) around the Pathogen.
- (iv) Forms a vesicle-like structure called ~~Phag~~ Phagosome.
- (v) Phagosome fuses with another vesicle called Lysosome.
(Lysosome has anti-microbial / hydrolytic enzymes.)
- (vi) The fusion is called phago-lysosome. It will degrade Pathogens into small molecules.





NOTE : OPSONISED PATHOGENS (pathogens that are surrounded by Abs or complement proteins) are more prone to recognition & degradation than free pathogens.

Dendritic cells → Activation of Adaptive immunity
most efficient in

- Phago-lysosome :
- It has several Anti-microbial Proteins & Peptides.
 - It also has a lot of hydrolytic enzymes that gets activated upon low pH
 - Oxidative Attack - increased formation of ROS (Reactive Oxygen Species) & RNS (Reactive Nitrogen Species). They are highly oxidative molecules that will oxidise biomolecules of the Pathogen.

Eg: Oxidation of di-sulphide bridges in Poly-peptides.

→ NADPH oxidase : Activation upon binding of PAMP with PRR.

→ It oxidises Oxygen to its super-oxide radical (O_2^-)

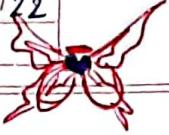
→ Super-oxide ~~radical~~ is converted to hydrogen peroxide by superoxide dismutase

→ H_2O_2 is converted into hyperchloric acid by myeloperoxidase

→ iNOS : Inducible Nitric oxide synthase. It's also activated by PRR binding.

→ It first, form nitric oxide (from AA residues)

highly oxidative oxidising



RNS

→ Nitric oxide reacts with superoxide radical to form a radical called **Peroxynitrite** (ONOO^-)
highly ~~nitrate~~ oxidizing

PRRs can also recognise **DAMPs**.

DAMPs: Damage associate molecular patterns

↳ Present on necrotic / APOPTotic host cells.

PRRs -

4 categories: **Toll-like receptors** (TLRs), **C-lectin receptors** (CLRs), **RLRs**, **NLRs**

NOD-like receptors

NOTE: Among the 4, TLRs are the most characterised ones.

TLRs: {TOLL-LIKE RECEPTORS}

→ 13 types: TLR 1 → found in human only

→ Human only TLR 11-13 → found in mouse only

→ Most of the TLRs form homodimer upon binding (except for TLR 1, TLR 2, TLR 6)

TLR 1, TLR 2 & TLR 6 forms heterodimers with each other. (TLR 2:6 / TLR 1:2)

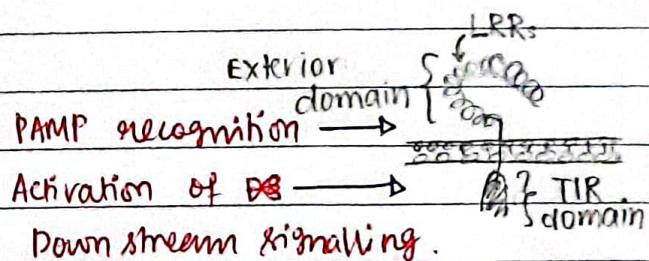
→ TLRs are found in most innate immunity cells.

→ TLR 1, 2/1, 2/6, 5 & 11 are present in the plasma membrane.

→ TLR 3, 7, 8 & 9 are present intracellular, embedded into

extracellular
pathogen
recognise the organelles (Endosomes).

recognise
intracellular
pathogen



LRR - leucine-rich repeats

TIR - toll-interleukin receptor

TLR Signalling Pathway -

- PAMP binding to PRRs
- Dimerisation of receptors
- Activation of TIR domains
- Recruitment of Adaptor molecules & docking in TIR domain
- ~~two types of Adaptor Protein~~ — **MYD88** [Myeloid Differentiation factor]
most TIR receptor bind to it
- **TRIF** [TIR-domain containing Adaptor-inducing IFN- β Factor]
TLR 3 will bind to it.

TLR 4 (in Plasma membrane) → binds to MYD88.

TLR 4 (in Endosomes) → binds to TRIF.

→ {TRIF & TRAM} increases binding affinity of the MYD88 & TRIF proteins.

Accessory Adapter molecules Activation of NF- κ B translational factors & MAP-kinase pathway

→ AP-1 & NF- κ B will code for the antimicrobial peptides, pro-inflammatory molecules (like ~~receptors~~, IFN- α , IFN- β)

→ TLR 7 → recognise ss RNA from virus

TLR 9 → ^{recognise} dsDNA from bacteria & virus.

They will meet AD Adaptor molecules TLR-8.

→ MyD88 can also help in activation of another activation factor called IRF (Interferon Regulatory factors).

→ **IFN- α , IFN- β** are secreted by IRF, NF- κ B & AP-1

TYPE I

NOTE : IFN- γ is not secreted (despite being virally infected cells) because they are secreted only by T-cells & NK cells. However, these cells are Macrophages.

activates Pathway

~~TLRs~~ TLRs present in RM. ~~secreted~~ → AP-1, NF- κ B

~~TLRs~~ TLRs present in Endosome ~~secreted~~ → AP-1, NF- κ B, Interferons
Activates Pathway

TLR (in PM) → Activation of AP-1, NF-κB Pathway

TLR (in Endosome) → Activation of AP-1, NF-κB Pathway + Secretion of IFN.

Diff. Cytokines that are expressed upon activation of AP-1 & NF- κ B

Pathway - (i) IL-1 β , TNF- α , IL-16 (Pro-inflammatory cytokines)
(ii) CXCL8 (Chemokine - T cell recruitment)

(iii) IL-12 (NK cells Activation, induces differentiation of CD4 T cells to $T_{H}1$ cells).

11/03/22 Inflammation:

Characteristic features -

- Swelling → Heat
 - Redness → Pain

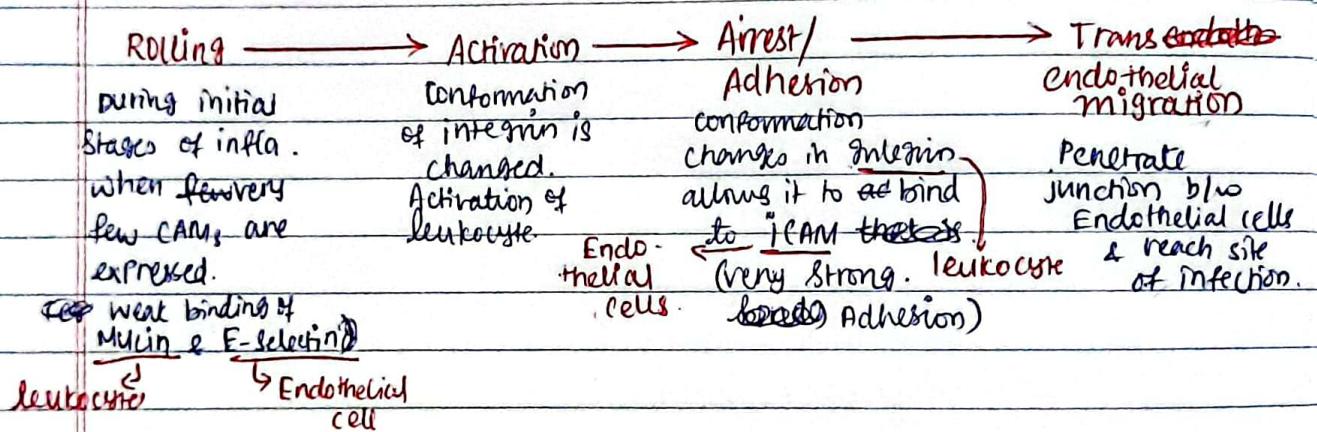
Within mins of pathogenic invasion -

- The tissue-resident macrophages recognise pathogens with the help of their PRRs.
 - Activation of transcriptional factors
 - Cytokines are secreted
 - Vasodilation : Rise of blood volume to area of infection
 - ↳ triggered by the cytokines
 - Increased vascular permeability that leads to accumulation of fluid present in the blood to the area of infection
 - ↳ cause of edema (swelling)
 - Leukocytes adhere to endothelial cells → blood vessel lining.
 - ↓
 - Extravasation
 - cause of pain
 - Due to increased activity near area of infection, the temp rises
 - cause of heat

NOTE: Neutrophils are first recruited during inflammation

Extravasation:

- Adhere Leukocytes starts to adhere to endothelium cells of the blood vessels.
- Increased expression of Cell Adhesion Molecules (CAMs).
- penetrate walls of blood vessels to move to the site of infection.



Acute Phase response -

- Pro-inflammatory cytokines: IL-1, TNF- α , IL-6
- These 3 cytokines induce the liver to secrete Acute Phase response Proteins.
- There are 3 types-
 - CRP : C-reactive protein
 - MBL : Mannose binding lectin
 - SF-A, SF-B : Surfactants - A, B.
- These Proteins help in activation of complement system
- The ~~with~~ complement Proteins binds to the pathogens & increase the Phagocytosis of the antigen → opsonization
- Apart from inducing complement system, these Proteins can also act as opsons (opsonization of pathogens)

Harmful effects -

- **Sepsis:** It is a condition seen in gram -ve bacterial infection. The LPS in the cell wall is recognised by TLR 4. This interaction is very very strong which leads to increased secretion of pro-inflammatory cytokines. This makes condition of inflammation chronic in nature which leads to damage of healthy tissues. *Since fluid is moving out* **It significantly reduces the blood pressure** leading *blood vessel into the infection site* to reduced blood flow to ~~the~~ organs (Septic shock) that can lead to multiple organ failure.
- **Rheumatoid Arthritis:** There is ~~at~~ prolonged activation of innate immune system due to accumulation of uric acid crystals in the bone joints.

14/03/22 Natural Killer Cells :-

Lymphocytes with innate immune functions.

Targets: Tumour cells, virally-infected cells

Cytokines that activate NKC: Interferons.

Mode of Action: Neutralization or Release of cytokines that recruit Tc cells.

When NKC receptor recognises infected cells, it will release

Perforin & **Granzyme B** ~~protease~~ enzymes.

↳ creates pores : Induces lysis of infected cells.

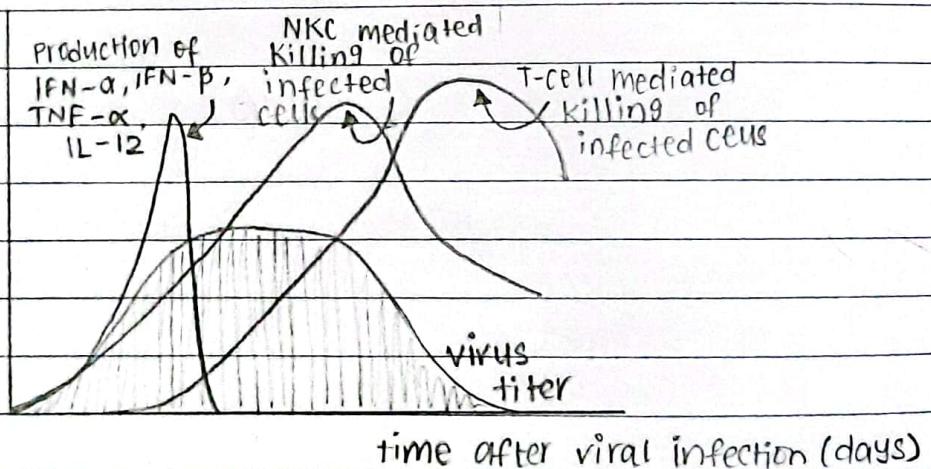
↳ Protease: cleaves inactive Pro-apoptotic Proteins making them active & inducing apoptosis in infected cell

NOTE: NKC control viral replication but not elimination.

↳ since rate of replication of virus is higher than the rate of elimination by NKCs.

virus titer → viral load

- during initial days of infection, the viral load is less. Release of cytokines such as Interferons & Pro-inflammatory cytokines.
- These cytokines will activate NKCs.
- Once NKCs gets activated, the viral titer flattens.
- NKCs are very effective in controlling viral ~~con~~ replication but can't effectively remove the virus.
- The viral titer dips when the Tc cells are activated.



Relationship b/w Innate & Adaptive immune system:

w.r.t Dendritic cells + cell

↳ the most effective APC.

- Dendritic cells also possess PRRs.
- Dendritic cells gets activated upon interaction with antigens.
- induction of pathways.
- Cytokines are released upon gene expression
- These cytokines recruit adaptive immune cells to dendritic cells
↳ naïve T cells.
- Upon interaction of T cells with Antigens Present by the APC (here Dendritic cells), T cells gets activated & differentiates

into TH, Tc or **Tr** cells. ↗ Differentiation depends on the type of TLR that interacts with the PAMPs.

↳ Inhibitory role: Removal of excess immune cells after cleaning infection; also controls inflammation

Research Study :-

Helminth produces several proteins that suppress the innate & inflammatory responses in the host.

One such protein is ES-62.

A research group extracted ES-62 protein from helminths to be used to reduce inflammatory response in septic shock syndrome. They also wanted to analyse its mode of action.

Model system: Macrophages (from patients with sepsis)

They performed 3 diff. treatments -

(i) Treatment 1: ~~Kef~~ Negative control

The Macrophages weren't treated with anything.

(ii) Treatment 2: Positive control

Macrophages were exposed to **molecules** that is known to induce septic shock. LPS.

(iii) Treatment 3: Treatment with ES-62 and LPS in the presence of LPS.

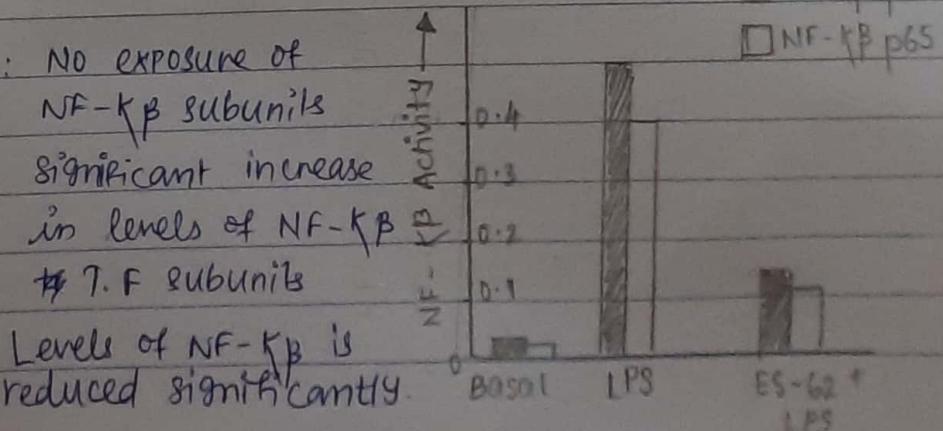
Analysis -

→ Checked levels of NF- κ B subunits ($p50$, $p65$) using real-time PCR.

- Negative control: NO exposure of (Basal) NF- κ B subunits

- Positive control: Significant increase in levels of NF- κ B subunits

- ES-62 + LPS: Levels of NF- κ B is reduced significantly.



→ Checked levels of pro-inflammatory cytokines.
Similar results to that of NF-κB.

Conclusion: ES-62 significantly reduces levels of pro-inflammatory cytokines and the pathways that induce its release.

Mode of Action Check -

Check if ES-62 competes with LPS for TLR-4 receptor.

Result: It is ~~not~~ independent of binding with TLR-4
(in fact, any TLR).

It rather ~~affects~~ affects a molecule involved
in the signalling pathway.

~~MYD88~~ Target → MYD88

Method → ~~using~~ Western Blot at 0, 3rd, 6th hour

Headings of macrophages in the presence
of ES-62.

NOTE: House-keeping genes (such as α -tubulin) are used as positive controls in western Blots since their levels of expression is always constant.

Observation → ES-62 degrades MYD88.