Overview of IMMU3903

- L1 – Immunity to Infection 1: Viruses and Immune evasion
- L2 – MHC/Genetics of Immunological Disease
- L3 – Immunity to Infection 2: Extracellular bacteria and Immune evasion
- L4 – Immunity to Infection 3: Intracellular bacteria and Immune evasion
- L5 – Immunity to Infection 4: Parasites and Immune evasion
- L6 – Vaccination and Novel Vaccine Approaches
- L7 – HIV and AIDS
- L8 – Self-tolerance
- L9 – Molecular Basis of Primary Immunodeficiency
- L10 – Allergic Diseases: Allergy and IgE-mediated hypersensitivity reactions
- L11 – Autoimmunity
- L12 – Imaging the Immune System
- L13 – Tumour Immunology
- L14 – Mucosal Immunology
- L15 – Transplantation Immunology
- L16 – Skin Immunology
- L17 – Neuroimmunology

LECTURE 1 (IMMUNITY TO INFECTION 1: VIRUSES AND IMMUNE EVASION)

Review the principal mechanisms of protective immune response to viruses
Innate host immune responses to viruses
Adaptive host immune responses to viruses
Viruses need to evade our immune system to survive
Example of how viruses fight back and disarm the innate and adaptive immune responses

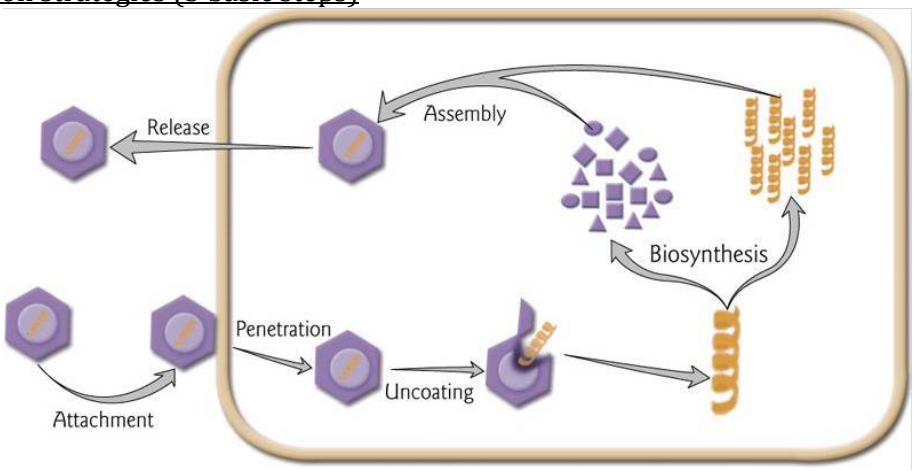
Virus

- Consists of proteins and nucleic acid
- Obligate intracellular pathogen → unable to replicate independently of a host intracellular machineries
- >400 viruses can cause diseases in humans

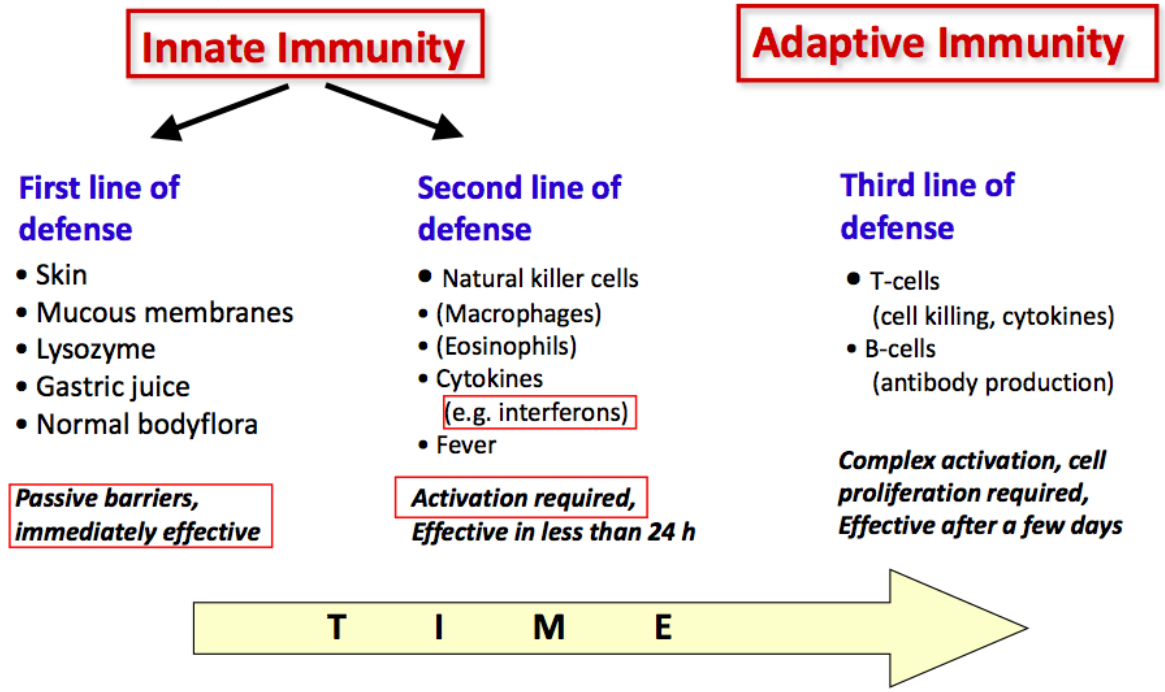
Routes of viral entry

- Skin
- Respiratory tract
- GIT
- Genitourinary tract
- Conjunctiva

Viral replication strategies (3 basic steps)

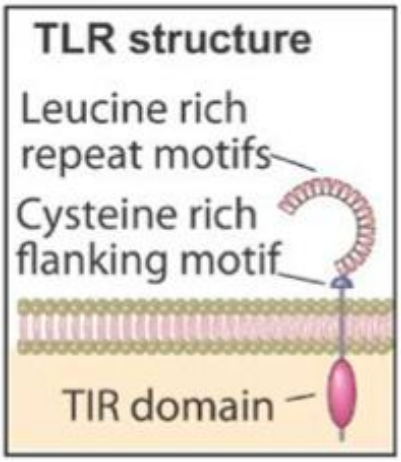


1. Attachment + delivery of genome into host cell (breach host barriers)
2. Hijack host cell transcription + translation machineries → use host cell's building blocks to copy viral genomes and synthesise viral proteins
3. Viral genomes and proteins are assembled and exit host cells as new infectious virus



Innate vs Adaptive immunity

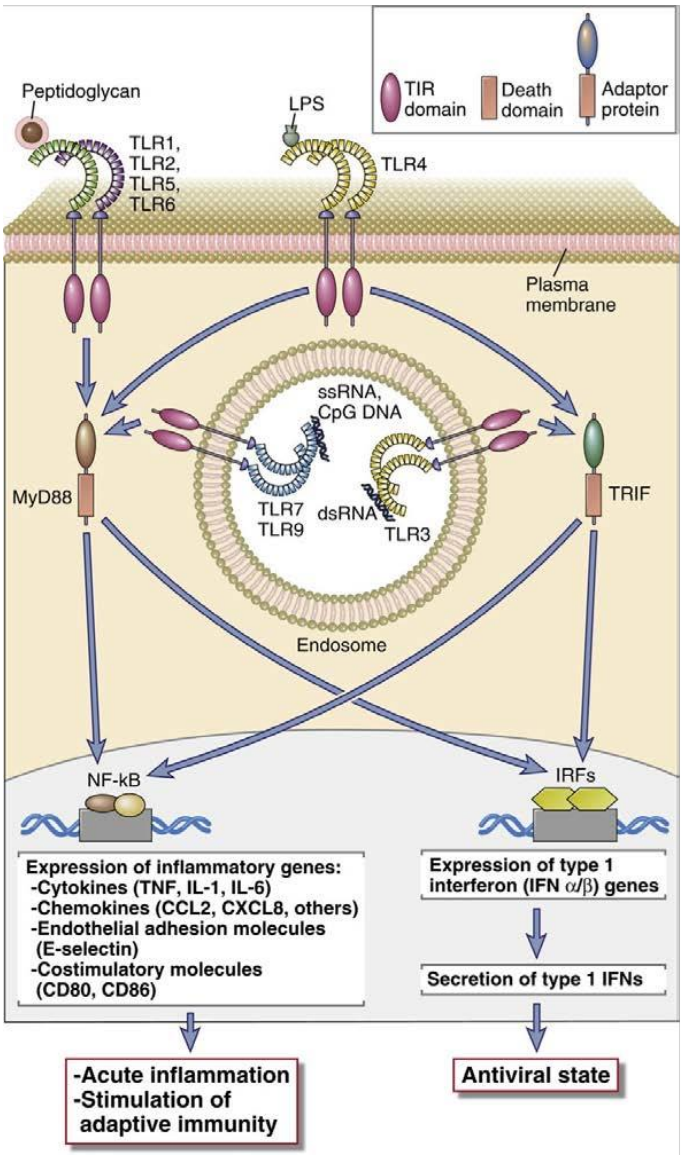
	Innate	Adaptive
Specificity	Recognise PAMPs shared by classes of microbes and DAMPS	Recognise microbial antigens and may recognise non-microbial antigens
Receptors	PRRs encoded in germline → limited diversity	Encoded by genes produced by somatic recombination (VDJ) of gene segments → give rise to greater specificity
Distribution of receptors	**Non-clonal** = identical receptors on all cells of the same lineage	**Clonal** = clones of lymphocytes with distinct specificities expressing different receptors
Discrimination of self/non-self	Yes → healthy host cells are not recognises (may express molecules that prevent innate immune reactions)	Yes → based on elimination or inactivation of self-reaction lymphocytes (may be imperfect → autoimmunity)



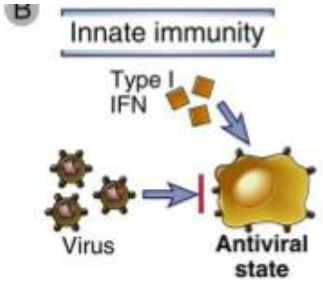
Virus-specific TLRs (endosomal)

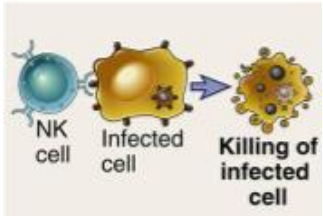
- TLR3: dsRNA
- TLR7/8: ssRNA
- TLR9: unmethylated CpG DNA

Signalling pathways and functions of TLRs



Innate Immunity to Viruses



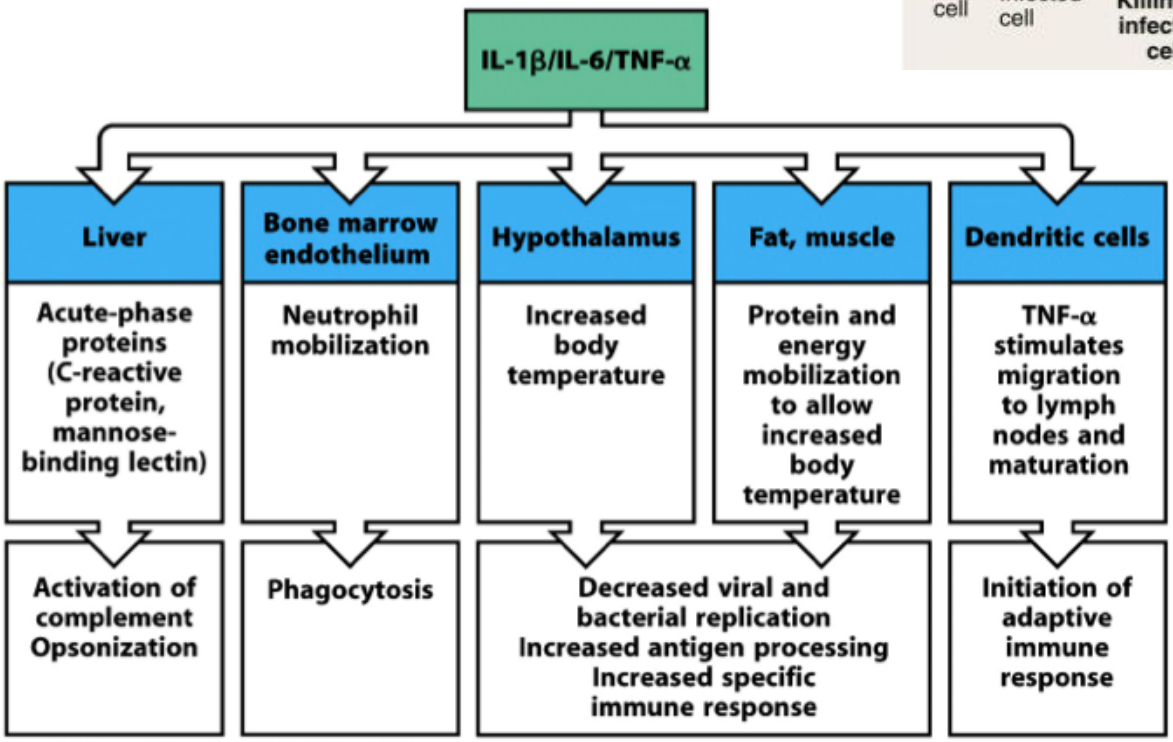


Principle mediators of innate immunity are:

- Inflammatory mediators (cytokines)
- Type I interferons (IFN-α, IFN-β)
- NK cells

Cytokines

- Low MW proteins secreted in response to pathogen
- Regulators of innate and adaptive immunity
- Extremely potent
- Production is tightly regulated
- Wide spectrum of biological activities



Type I Interferons (IFNs) → anti-viral

- Mediate early innate immune response to viral infection

Function

a) Inhibit viral infection and replication by inducing anti-viral state
b) ↑ MHC class I expression and Ag presentation in cells
c) Activate NK cells → selectively kill virus-infected cells

Induction of type I IFNs

Endosomal viral components	Cytosolic viral components
• ssRNA → TLR7/8 • dsRNA → TLR3 • Unmethylated CpG DNA → TLR9	• Short ss/dsRNA → RIG-I • Long dsRNA → MDA-5 • dsDNA → DAI (DNA-dependent activator of IRFs)

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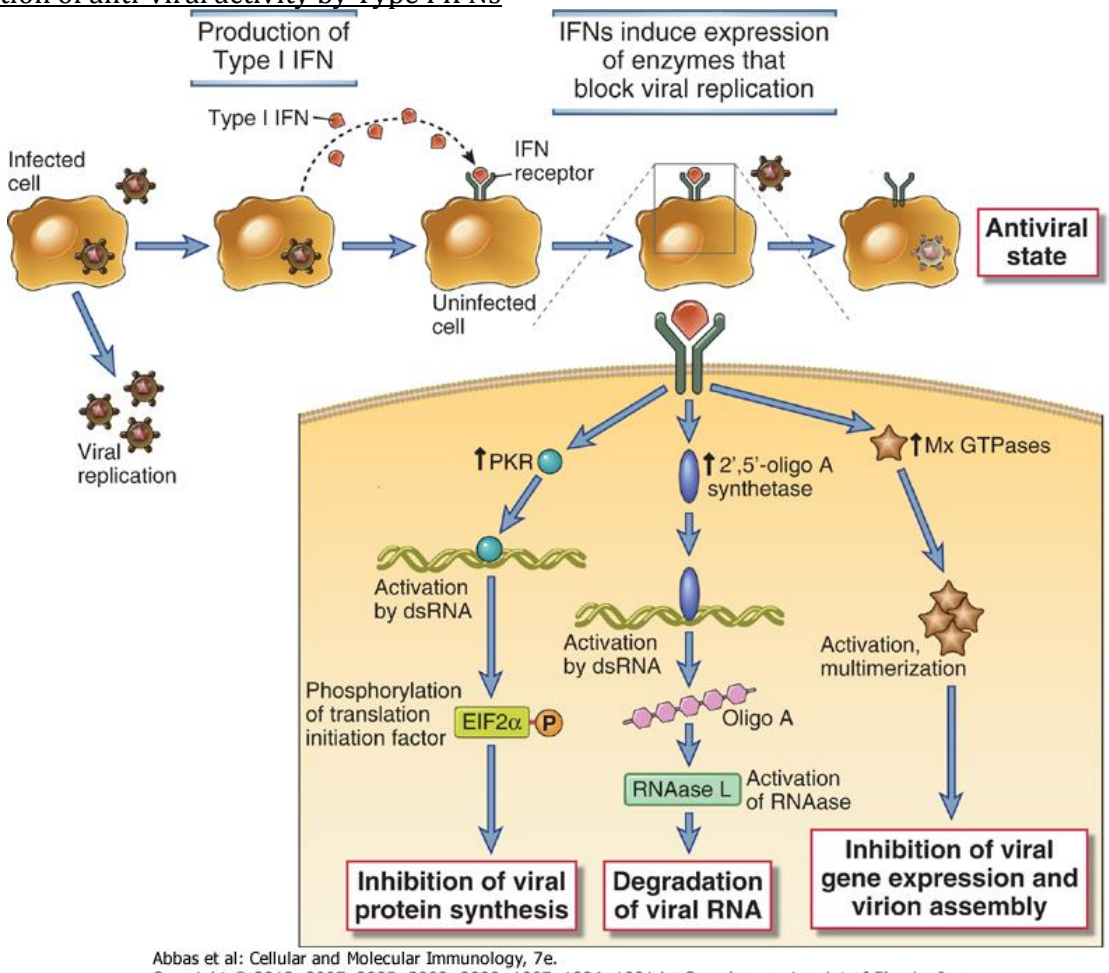
Activation and translocation of IRFs (IRF1/3/7) into the nucleus

↓

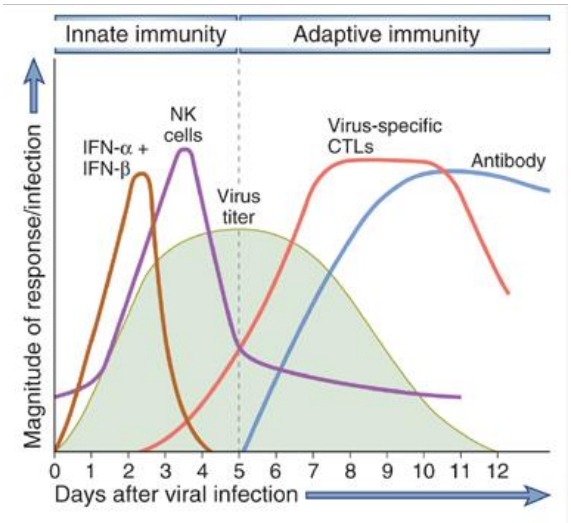
↑ transcription of IFN-α & IFN-β

Induction of anti-viral activity by Type I IFNs



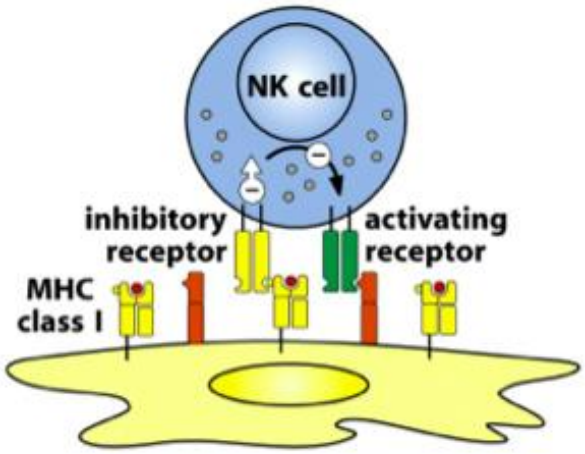
- **PKR (eIF2α kinase)** → phosphorylate of eIF2α → inhibition of viral protein synthesis
- **2'-5'-oligoA synthatase** → activate latent RNAse → degradation of viral RNA
- **Mx GTPases** → inhibits viral gene expression and virion assembly

NK cells



- Subset of lymphocytes LACKING Ag-specific surface receptors
- Contain cytotoxic granules
  - Can kill tumour and virus infected cells
- Comprise 5-15% of the mononuclear cells in blood/spleen
- Express **CD16** and recognise IgG-bound cells → activate **ADCC**
- Recognise a reduction in cell-surface MHC class I
  - Almost all nucleated cell-types express MHC class I
  - Viruses downregulate MHC class I → become target for NK cells
- NK cells function early in the course of virus infection
  - Sit within the interface between our innate and adaptive immunity
  - Kill virally infected cells before Ag-specific CTLs become fully active

To control their cytotoxicity, NK cells express both activating and inhibitory receptors

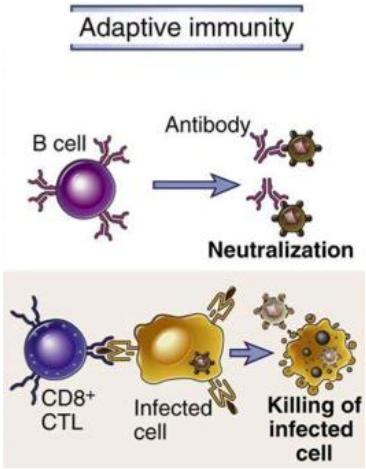


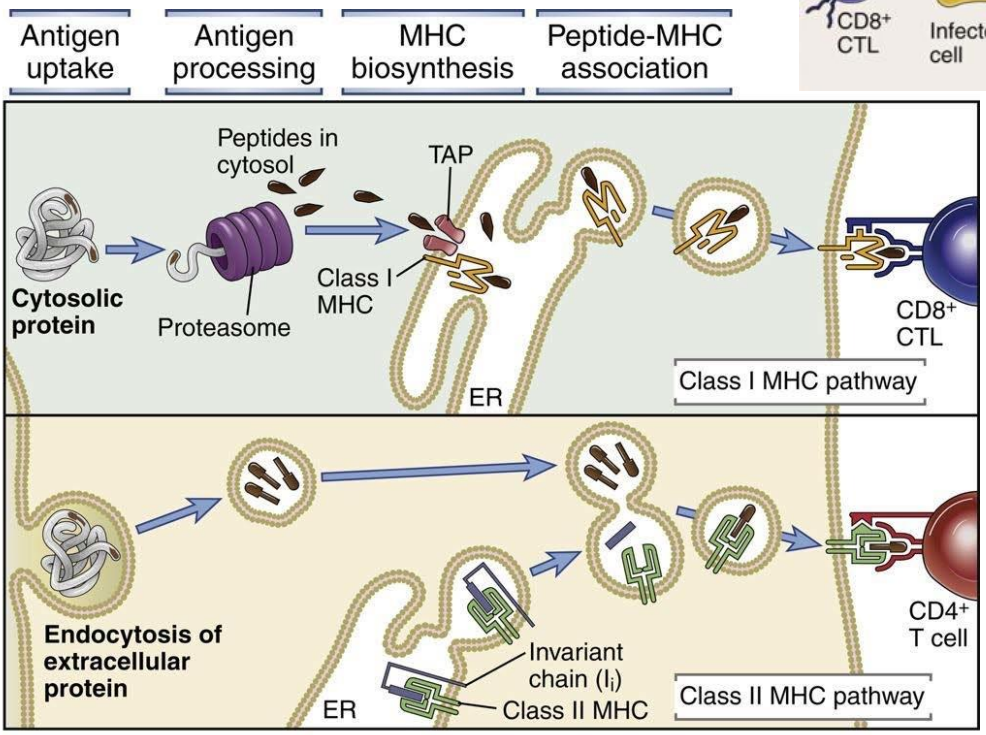
- Activating receptors → recognise NK cell activating ligand on cells
- Inhibitory receptors, i.e. Killer-cell immunoglobulin-like receptors (KIRs) → recognise MHC class I on normal cells → inhibit activating receptor signals by stimulating a negative signal → no kill
    - However, **viruses downregulate/alter MHC class I** → inhibitory receptor no longer bind/recognise → **NK cells activated by activating signals** → release cytoplasmic granules to induce target cell apoptosis

Adaptive Immunity to Viruses

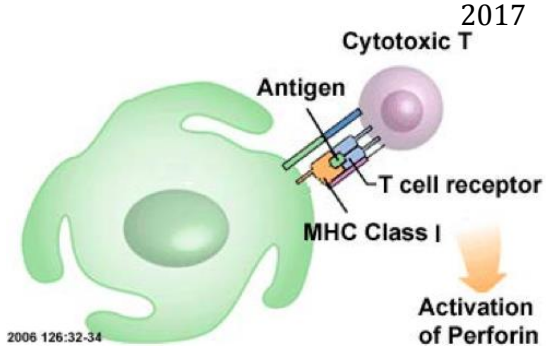
Adaptive immune response against viruses is mediated by:

- CTLs
- Helper T cells
- Antibodies





Cytotoxic T lymphocytes (CTLs)



- Principle effector cells in antiviral immunity
- Eliminate intracellular viruses via lysing of infected cells
- CD8+ CTLs recognise antigenic peptides presented by **MHC class I**

Activation of naïve CTLs

- Naïve CTLs unable to perform effector function
- Require 2 signals to be activated (provided by APCs in lymphoid organs)
  - MHC-peptide complexes
  - Co-stimulators

Signal	T cell	APC	Description
First Signal	TCR	peptide-bound MHC class I molecule	There is a second interaction between the CD8 coreceptor and the class I MHC molecule to stabilize this signal.
Second Signal	CD28 molecule on the T cell	either CD80 or CD86 (also called B7-1 and B7-2)	CD80 and CD86 are known as *costimulators* for T cell activation. This second signal can be assisted (or replaced) by stimulating the T_C cell with cytokines released from helper T cells.

Cross-presentation

- Ability of certain APCs (primarily DCs) to take up **EXTRACELLULAR** antigens and present them on MHC class I to CTLs
- Necessary for immunity against viruses/bacteria/tumours that <u>does not</u> invade APCs

Endocytosed proteins are transported out from the endosome into the cytoplasm

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Processed by the proteasome into peptides

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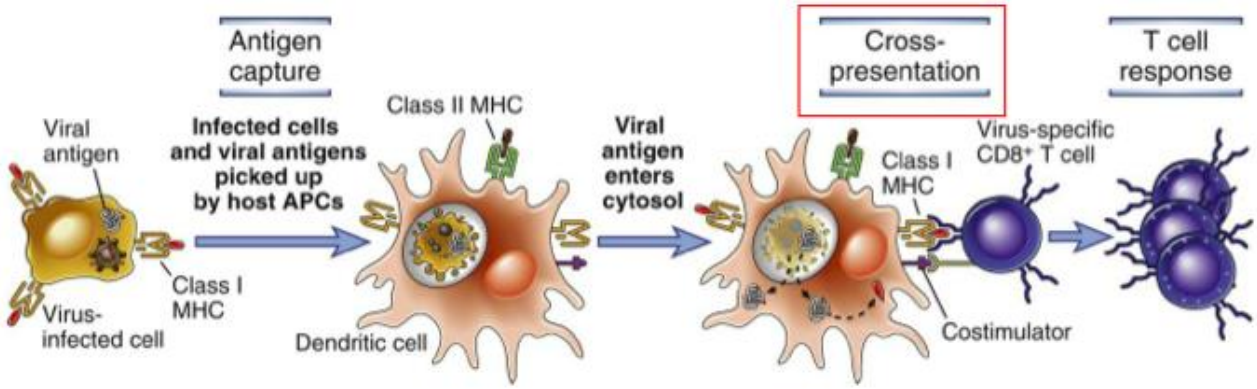
Transported by TAP transporter into the ER where they associate with MHC I



CTL killing of infected target cell

After the formation of the immunological synapse, CTL realign its cytotoxic granules towards the immunological synapse

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<u>Release of perforin and granzymes</u>

↓

Granzymes enter cell via receptor-mediated endocytosis

↓

Granzymes enter cytoplasm via Perforin-dependent mechanisms

↓

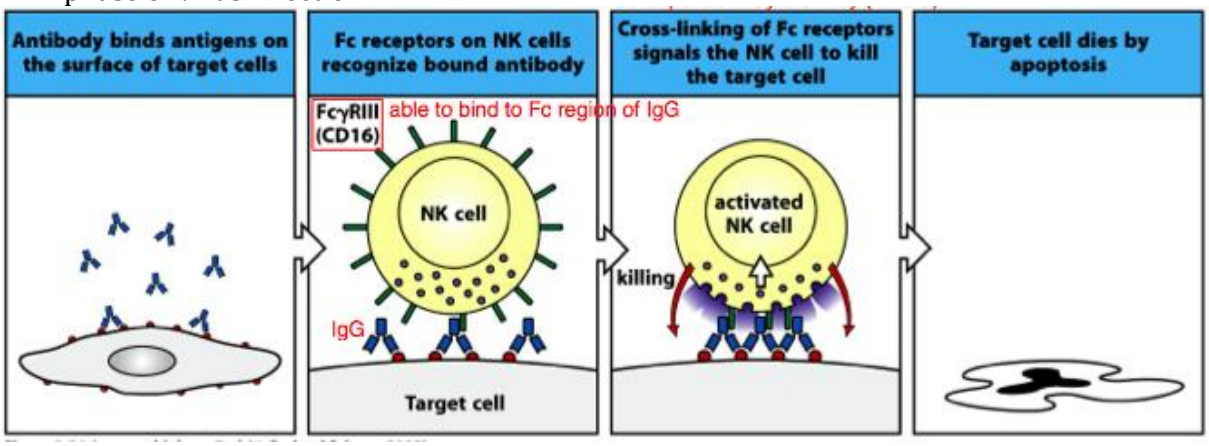
Activation of apoptotic pathways

Helper T cells

- CD4+ T cells → important in providing help for the Ab, CTL and inflammatory responses via secretion of cytokines
- Recognise antigenic peptides presented by cell-surface **MHC class II**

Antibodies (Abs)

- Recognise epitopes (part of Ag to which Ab attaches itself)
- Effective against viruses primarily during the EXTRACELLULAR phase of their lifecycle
  - **Neutralising Abs** → prevent virus binding to its cell-surface receptor
  - **Opsonising Abs** → opsonise viral particles to promote clearance by phagocytes
- **ADCC (Ab-dependent cell-mediated cytotoxicity)** → effective during INTRACELLULAR phase of virus infection



How viruses evade immune recognition

1. Avoid recognition by Ab
2. Hide from the immune system
3. Directly infect and destroy/alter cells of the immune system
4. Affect the response and activation of the immune response
5. Produce "dummy" decoy immune molecules

1) Viral evasion of Ab responses

- Avoid Ab detection and thus neutralisation

Antigenic drift

- Small changes/point mutations in viral genes → alter structure of viral surface proteins
- Require new immune response to control virus

Antigenic shift

- Obtain new genes from another genetically different virus → change in structure of viral surface proteins → new virus subtype (require new immune response)

2) Hide from the immune system

- Infect "immune privileged" sites → avoid Ag presentation
  - Infection of neurons by HSV and VZV (these infections are never cleared)
- Establishment of latent "dormant" infection
  - HSV/hCMV → limited/no viral Ag expression → no immune recognition

3 phases of HSV/hCMV infection

a) Productive → Lytic gene expression + release of infectious virus + retrograde tp to establish latency
b) Latent (lifelong state) → viral genome present in the cell w/o viral gene expression
c) Reactivation → virus reawakens from latency → new progeny infectious virus

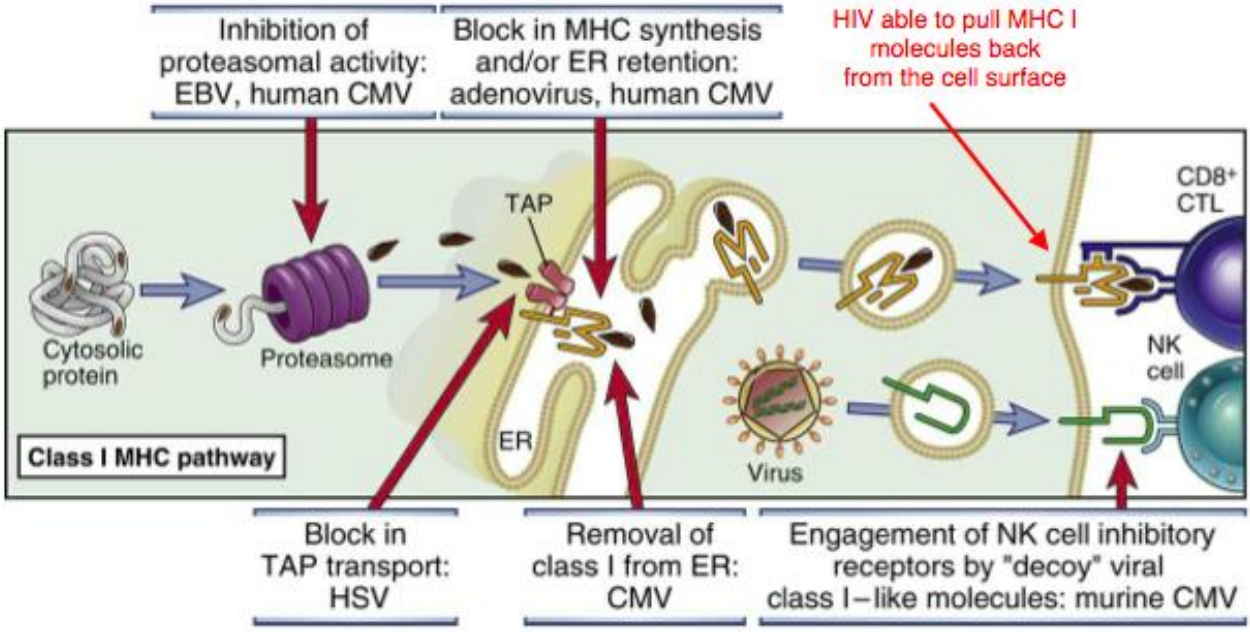
3) Infect and destroy cells of the host immune system

HIV infects + depletes human CD4+ T cells (critical to adaptive immune response) via:

- Replicating in CD4+ T cells → cause cell lysis upon virus exit
- Induction of apoptosis
- Trigger CD8+ T cell recognition and lysis of infected cells

4) Affect the response and activation of the immune response

- Expression of immunomodulatory proteins → alter immune response
  - Able to interact with components of both the innate and adaptive immune responses
- Regulate cytokines → modulate communication between cells
  - Production of virally-encoded immunosuppressive cytokines
  - Disable immune communication
    - Intracellular inhibitors of Type I IFN signalling
      - Modulate PKR → allow viral protein synthesis
      - Modulate 2'5'-oligoA synthetase → prevent viral RNA degradation
- Manipulating MHC class I and class II pathways



5) Produce "dummy" decoy immune molecules

- **Receptor mimicry**
  - Poxviruses encode TNFR homolog → TNF-α binds to homolog instead of actual receptor → no pro-inflammatory response
- **Virus encoded cytokine homologs**
  - hCMV and IL-10
    - IL-10 (anti-inflame cytokine) = inhibitor of activated macrophages and DCs
      - Suppress MHC class II expression → infected cells cannot present viral antigen
    - Virally encoded IL-10 homolog → **enable virus to inhibit host immunity**

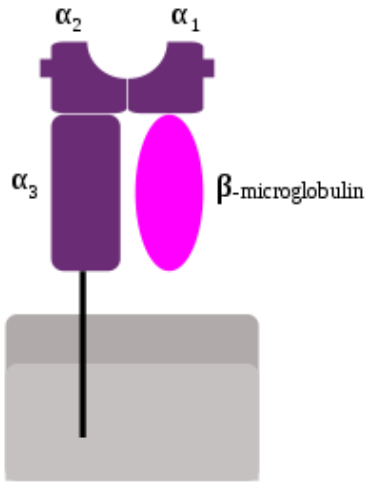
LECTURE 2 (MHC/GENETICS OF IMMUNOLOGICAL DISEASE)

Review of HLA system

Genetic restriction of T cell response

- Activation of TCR → Restricted by self MHC molecules presenting peptides
- Peptide **MUST** bind to HLA molecule to be able to activate T cell (MHC restriction)
- MHC class II → CD4+ T cells
- MHC class I → CD8+ T cells

Class I MHC molecules



Structure

- Heavy α chain (3 domains)
  - ***Variable α1 & α2*** → form **closed** peptide-binding cleft
  - ***Invariant α3*** → contain binding site for T cell co-receptor CD8
- ***β2 microglobulin*** (single domain), invariant

Types

- HLA-A
- HLA-B
- HLA-C

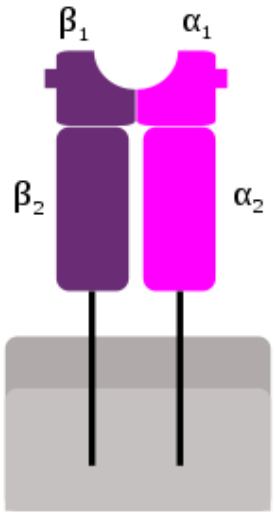
Distribution

- All nucleated cells

Binding

- CD8+ T lymphocytes

Class II MHC molecules



Structure

- **α chain** (2 domains)
  - Variable α1 → Form **open** peptide-binding cleft with β1
- **β chain** (2 domains)
  - Variable β1
  - Invariable β2 → contain binding site for T cell co-receptor CD4

Types

- HLA-DP
- HLA-DQ
- HLA-DR

Distribution

- DCs, monocytes, B cells and inducible in some cells with IFN-γ

Binding

- CD4+ T lymphocytes

Human Leukocyte Antigen (HLA complex)

- Most important region in controlling the immune response (but not the only region)
  - Encoding the 6 major antigen-presenting proteins (HLA-A etc.)
- Resides on a 3Mbp stretch within chromosome 6p21
- HLA genes are **highly polymorphic** (having many different alleles)
  - Able to tract migration of population around the world
  - Diversity in HLAs in human population is one aspect of disease defence

Patterns of genetic effects influencing immune response to infection

Pattern of inheritance

- Mendelian inheritance
  - Autosomal recessive
  - Autosomal dominant
  - X-linked
- Polygenic inheritance
  - **Quantitative trait locus** = region of DNA associated with a particular phenotypic trait → varies in degree and attribute to polygenic effects
  - Cumulative effect of SNPs (molecular markers) in multiple genes to influence an observed trait

Genetic spectrum & infection

Global deficiency ⇔	Single defect ⇔	Major allele ⇔	Polygenic
Absent T/B cell I.e. born without thymus	Lack particular type of T cells causing Mendelian susceptibility to mycobacterial disease (MSMD)	Spectrum of clinical leprosy symptoms dependent on QTL	• Many genes • Many SNPs I.e. HIV and TB

Genetic effects on immune responses

1) Monogenic effects

- Effect of *SINGLE* gene on immune response
- Gene mutation (<1% freq.) → lead to absent or non-functional protein
- Mendelian pattern of inheritance

I.e. Primary immunodeficiency

A. Global deficiency → absence of T or B cells
   a. T cell deficiency caused by absence of common gamma chain of IL-2 receptor
B. ↑ Susceptibility to specific class of microbes → uncommon response to common pathogen
   a. MSMD (Mendelian Susceptibility to Mycobacterial Diseases) due to deficiency in IFN-γ or IL-12 receptor

2) Polygenic effects

- Small effects of gene mutation *OR* allelic variation in *MULTIPLE* genes influences immune response
  - Often SNPs leading to loss-of-function/changes in function
- Individual gene may have Mendelian pattern of inheritance
  - I.e. require SNP in both genes to lose function (AR) or if protein acts as dominant negative mutant (AD)
- Complex → phenotype requires the effects of *MULTIPLE* variants

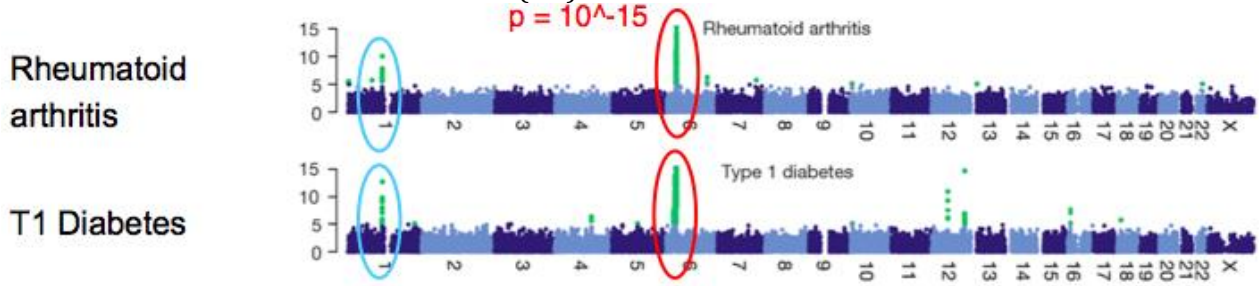
Predictions of polygenetic effects

A. **Family association studies** → risk associated with inheritance of one region, gene locus or allele
B. **Case control studies**
   a. Gene products (i.e. HLA genes) is associated with variation in immune responses & therefore type of human disease
      i. Variations include risk of infection/allergies/autoimmune response/vaccine response etc.
   b. Assemble group of cases and controls → measure freq. of variation in 2 groups

C. Genome wide association studies (GWAS)

a. Risk of disease associated with variation in SNPs across whole genome
b. Relative risk can be used as a negative risk prediction value
c. GWAS for common diseases → ID regions of disease-associated chromosome

Manhattan Plot of Rheumatoid Arthritis (RA) and T1D



- Found significance between genes associated with both RA and T1D on C6 and C1
  - C6 contains *HLA-DRB1* locus (MHC class II → regulate CD4+ T cells)
  - C1 encode *PTPN22* → "turns off" signalling in T cells and macrophages
  - Thus HLA class II variations strongly associated with both RA and T1D

HLA variation & autoimmune disease

I.e. Patients with **Ankylosing spondylitis (AS)** express HLA-B27+ mutation

- Early symptoms of AS include chronic pain + stiffness of the lower back (due to autoinflammatory response)
- Rel. risk of HLA-B27+→ 90-fold increase compared to HLA-B27- (association determined by case control studies)
- Negative risk prediction → pt present with lower back pain but HLA-B27- → unlikely to be AS

HLA association & drug reaction

I.e. **Drug-induced exfoliative dermatitis**

- Abacavir (anti-retroviral drug for HIV)
  - HLA-B57+ → much greater chance of drug-induced exfoliative dermatitis
  - Now test for gene by flow cytometry before drug use

Genetics of infectious diseases

Tuberculosis (TB)

- Aerosol transmission
- Multiple factors determine disease (nutrition, HIV, environment, **genetics**)

Genetic susceptibility to TB

- Ethnic variations (independent of socio-economic factors)
- MSMD
  - Rare AR condition with risk of TB development following the BCG vaccination due to mutations in the IL-12R and IFN-γR

GWAS in TB pts

- Association with *ASAP1* gene on chromosome 8
  - ASAP1 involved in actin & membrane remodelling
- SNP in ASAP1 → ↓ migration of DCs → less able to stimulate immune response to control TB

Leprosy

- Caused by *Mycobacterium leprae*
- Spectrum of leprosy (extent of disease severity) is controlled by immune response

GWAS of leprosy pts

- Found significant association in mutations of HLA-DR (MHC class II) and components of the Inflammasomes (NOD2/RIPK2)
- Shows the importance of HLA-DR and innate immunity in leprosy

Meningococcal disease

- Associated with risk of disseminated *Neisseria* infections
- **C5 complement deficiency** → prevent formation of Membrane Attack Complex

GWAS identified new associations with variations in Complement Factor H (CFH)

- CFH = inhibit formation of C3 convertase in Alt. complement pathway
- *N. meningitidis* avoids complement-mediated killing by recruiting CFH
- Variations in CFH determine invasiveness of disease

HIV

- Most common infective cause of immunodeficiency
- Environment + genetic factors

Transmission of many forms of HIV is dependent on the chemokine receptor **CCR5** and **CD4**

- CCR5 expressed on DCs and T cells

Genetic control of HIV infection

- **CCR5Δ32** (32bp deletion in CCR5) → truncated & non-functional → does not support infection of cells by HIV-1
- Homozygous (2 copies of CCR5Δ32)
    - High level resistance to HIV infection
- Heterozygous
    - Doesn't prevent infection BUT slower rate of disease progression

Genetic control of HIV progression

- HLA-B also affect rate of infections
- Some HLA-B haplotypes are more efficient at binding HIV peptides → effective stimulation of HIV-1-specific CD8 CTL