○ RIG-like receptors
  ▪ Cytoplasmic
  ▪ MDA-5 recognises double stranded RNA (dsRNA) from viruses
  ▪ Similar to TLR - redundancy
  ▪ African Horse Sickness
    □ dsRNA virus
    □ Infection increases mDA5 pathway signalling
  ○ PPRs very important
    ▪ Recognse conserved PAMPs
  ○ Different PPRs = different responses (different PAMPs)

Cells with PRR
  ○ Dendritic
  ○ Macrophages
  ○ Neutrophils
  ○ Eosinophils
  ○ Basophils
  ○ Mast cells
  ○ Natural Killer Cells
  ○ PPRs also on non-immune cells

  ○ Dendritic cells and macrophages are in tissyes
    ▪ "sentinel cells" - skin, liver, gut, lungs, etc.
    ▪ Range of PPRs to detect pathogens
  ○ Dendritic cells
    ▪ Langerhans in skin
    ▪ Range of PPRs
    ▪ Activation leads to inflammation
    ▪ Release of immune molecules
      □ Cytokines
        ◆ Small soluble
        ◆ Interleukins
        ◆ e.g. interleukin and interferons
        ◆ Bind to specific receptors on immune cells
      □ Chemokines
        ◆ Specialised cytokines - chemoattractant
        ◆ Attracts immune cells to tissues or within tissues
        ▪ Mediate immune responses
        ▪ Does not kill pathogens
        ▪ Takes in and presents pathogenic antigens
        ▪ Attract other immune cells
  ○ Macrophages
    ▪ Range of PRR
    ▪ Phagocytosis after PRR activation
    ▪ Can kill pathogens
    ▪ Critical for adaptive response

Killing
1. PRR and other receptors bind micropathogen
2. Transported by phagosome into cell
3. Fuse with lysosomes
4. Pathogen destroyed
   • Acification, oxygen derive toxins, antimicrobial peptides (defensins etc.), enzymes, competitors (lactoferrin sequesters iron)
   • Immune evasion
     □ Deliberate modulation of host immune system
     □ Capsules to prevent phagocytosis
- 1st lymphoid follicle - B cells
- Paracortical area - T cells
- Medullary cortex - macrophages
- Naïve lymphocytes constantly circulating
- Antigens and APCs move from infected tissues to lymph nodes

**Spleen**
- Filter microbes, antigens from blood **only**
- APCs and lymphocytes
- Same activation role as lymph nodes
- Red pulp
  - RBC destruction and storage - non-immune
- White pulp
  - Mature DCs and macrophages migrate from tissues to spleen via blood
    - Reside in marginal zone
  - Interact with lymphocytes in spleen

**MALT - Mucosa Associated Lymphoid Tissues**
- Gut Associated Lymphoid Tissues - GALT
- Nasal Associated Lymphoid Tissues - NALT
- Bronchus Associated Lymphoid Tissues - BALT
- Skin Associated Lymphoid Tissues - SALT
- Different environments
- Most exposure to pathogens at mucosal surfaces
- Higher lymphocytes concentration than the rest of the body
- Specialised lymphocytes
- Lots of IgA antibodies
  - Protected from mucosal proteases

**GALT**
  - Peyers patches
3. **Antibody-dependent cell-mediated cytotoxicity**
   - ADCC
     - Antibodies bind non-self antigens on the host cell
     - Immune cell Fc receptors bind antibodies
       - Host cell apoptosis triggered
       - Perforins etc.
     - Neutrophils, macrophages, eosinophils, NK cells

4. **Activation of Complement**
   - Antibodies 1 way of activating the complement cascade
   - Complement is a series of proteins in serum
   - Part of innate immune response
   - 3 pathways

```
Phagocytosis triggered

[Diagram showing the complement cascade]
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- **Classical Pathway**
  - Antibody-antigen complexes and some non-specific reacting
  - Initiated by C1q
  - Binds antibodies or pathogen surface
- **Lectin Pathway**
  - Lectin (PRR) molecules (not antibodies) bind pathogen surfaces
  - Initiated by mannose binding lectin or ficolins
    - Bind carbohydrates on pathogen surface
    - Mannose PAMPs on salmonella, fungi
- **Alternative Pathway**
  - Spontaneous reactivity at pathogen surfaces
  - Initiated by C3
  - Blocked on host cells by multiple proteins
    - e.g. CD59

- **Complement functions**
  - Destruction
    - Polymerisation of terminal proteins to form Membrane Attack Complexes
    - MACs form pores in cell membranes
    - Cell lysis
  - Opsonisation
    - C3b and C5a proteins induce phagocytosis
  - Inflammation

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• **Paracrine Action**
  - Act on nearby cells
  - E.g. IFN during a viral infection

• **Endocrine Action**
  - Act on distant cells
  - E.g. IL-1 in systemic inflammatory response

• **Pleiotropic Action**
  - Any given cytokine may have different roles depending on targets
  - E.g. IL-2, 4, 5 and B cell proliferation

• **Redundant Action**
  - 2 or more cytokines with similar functions
  - E.g. IFN-alpha and TNF - increased MHC1

• **Synergistic Action**
  - Combined effect of 2 cytokines is greater than the additive of each alone
  - E.g. IFN-gamma and TNF - increased MHC1

**Regulation of Cytokines**
- Non-specific function must be prevented
  - Transient function
• Also in heart and lung membranes

• Also impacts memory B cells
  ▪ Products bind and block TNFα
• TNFα inhibitors $22 billion in 2009
  ▪ Crohns
  ▪ Asthma
  ▪ Ankylosing spondylitis
    □ Spinal arthritis

• Anti-IL-2
  ▪ T-cell proliferation
  ▪ Transplant rejection
  ▪ Blocking antibody against IL-2
  ▪ Also used in MS treatment

• IL-2
  ▪ Cancer
  ▪ Ex-vivo application of IL-2 to lymphocytes
  ▪ Stimulated and activated anti-tumour response
- Repeated subpassage mutations accumulate
- Test for paralytic activity
- Clinical trials
- Mutations sequenced

Immune response
- Strong, appropriate response
- Cellular immunity
- Humoral immunity (including secretory IgA)
- Long-lasting memory

Advantages
- Multiple antigens
- Few immunisations
- Easy to produce without genome
- May not require adjuvant (modifier)

Issues
- Reversion to wild type (polio 2 and 3)
- Persistent infection (Varicella-zoster, chickenpox, shingles etc.)
- Severe case if immunocompromised (measles)
- Hypersensitivity to egg antigens (mumps)

2. Killed vaccine
- Killed by heat - can denature too many protein antigens
- Killed by chemical - formaldehyde (Salk polio)
- e.g. yearly flu vaccination, hepatitis A

Immune response
- Weaker response than live vaccines
- Good serum antibody response, little secretory IgA
- Poor cell-mediated immunity
- Booster shots usually required

Advantages
- Multiple antigens
- Stable
- Safer than live vaccine
- No refrigeration (attenuated can need this)

Issues
- Vaccines not always killed (polio)
- Lack of understanding about why it protects
- Contamination with animal viruses (polio)
- Initial preparation requires working with pathogen

3. Subunit and Toxoid Vaccines
- Specific, purified pathogen subunit/molecule
- Toxoid vaccines induce antibodies against the exotoxins
  - Exotoxins cause major symptoms
- e.g. tetanus, diphtheria
- E.g. strep. Pneumoniae, hepatitis B
- Immune response
  - Weak immune response
  - Good serum antibody response
  - No cell-mediated immunity
  - Booster shots usually required
- Advantages
  - Limited antigens - less chance of cross-reactivity
  - Higher levels of specificity and reproducibility
  - Safe than live no chance of accidental infection
  - No need for refrigeration
- Issues
  - Toxoid vaccine
    - Limited to few bacterial diseases
  - Limited number of antigenic targets - evolution
  - Difficult to develop
  - Ajuvant required
**Acute Phase Proteins**
- Haptoglobin
  - Binds haemoglobin
  - Prevents bacteria gaining iron
- Fibrinogen
  - Potential damage to tissues
  - Generates fibrin threads
  - Clot can block pathogen spread

**Resolving Inflammation**
- Malnutrition
- Cancer
- Drug treatment
- Organ removal
- Infection
- Stress
- Age

○ Primary = absences, secondary = reduced
  - Low T cell count
  - Lower B cell proliferation

○ Malnutrition
  - Different nutritional deficiencies --> different immunodeficiencies
  - Obesity also associated with cancer, inflammation, autoimmune diseases (rheumatoid arthritis)
  - Zinc - critical in T cell activation
    - Pigs with Zinc deficiency: decreased T, activity, B cell activity, NK activity, phagocytic activity
  - Copper deficiency also has impact
  - Vitamin A

- Deficient Vitamin A
  - Retinoic acid
  - NK cells
    - Reduced activity
  - T cells
    - Reduced proliferation
  - B cells
    - Reduced Ig production

- Infection
  - Select bacterial, protozoal, and helminth pathogens can impact immune responses
  - Major infections are viral
  1. HIV
    - Mainly sexual transmission
    - ~30 million infected
    - Infects CD4 T, cells
    - Also dendritic and macrophages
    - GP120 binds CD4, then CCR5 or CXCR4 (chemotaxis receptor)
Reproduction and the Immune System

- The mammalian immune system will reject non-identical tissues/cells of the same species
- Allograft rejection
- Blood transfusion
- Haemolytic disease of the newborn
- Every foetus is at least 50% maternal derived
  - Up to 50% non-self
- 3 main challenges
  1. Survival of male gametes in the reproductive system
  2. Implantation and development of the foetus
  3. Survival of the newborn after birth

Sperm and the Immune System

- Non-self
- Female reproductive tract is site of infection
- Not all survive
- 2-3% of women develop anti-sperm antibodies associated with subfertility or infertility
  - Hypersensitivity even rarer
- Various mechanisms that reduce the immune response
  - Seminal plasma
    - High TGFβ (T_{reg}), IL7 (immunoregulatory), IL8 (chemotaxis and phagocytosis) - not only regulatory
    - Regulatory prostaglandins
    - Lenicov et al 2012 - DCs cultivated with seminal plasma become tolerised, less inflammatory response
  - Sperm also provide protective mechanism
    - No MHC1 so should be NK target
    - Pang et al 2007
      - Sperm coated with glycans that reduce NK cell cytotoxicity
      - Also present on some cancer
      - Related to interactions of HIV

Foetus and the Immune System

- Implantation and development
- 1/2 chromosomes from father
- Specifically paternal MHC molecule
- Graft experiments show that uterus can reject non-self tissue
- Placental development linked to immune response
- Various mechanisms to reduce response
  - Not known if they’re present in all mammals
  - Likely to be differences
  - Human and mouse studies predominate
  - T_{reg} cells
  - Uterine NK cells
• Passive immunity
  • Haemochorial - humans, rabbits, rats, mice
    ○ 3 layers, embryo derived
    ○ Full antibody transfer
  • Endochorial - cats, dogs
    ○ 4 layers, 1 maternal derived, 3 embryonic
    ○ 6-10% IgG transfer
  • Epitheliochorial - ruminants, horses, whales
    ○ 6 layers, 3 maternal, 3 embryonic
    ○ No antibody transfer
• Different levels in colostrum and milk

![Diagram of antibody distribution in different species](image)

• Chicken passive immunity
  ○ IgY (ancestral E, G), IgM, IgA in serum
    ▪ Hen sera --> egg yolk (IgY)
    ▪ Oviduct --> albumin (IgA, M)
  ○ Protects 10-20 days
• Maternal Abs impact vaccination of early animals - 1/2 life 5-10 days
  ○ Inhibits newborn ability to respond
  ○ No maternal Ab in calves - 1 week to make Abs
  ○ Maternal Ab in calves - 4 weeks to make Abs
  ○ Cats and dog vaccinated after 8 weeks
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