MODULE 1: B Cells and Diseases

Antibodies

- Produced by differentiated B plasma cells
- Identify and neutralise foreign pathogens
- Specific to a particular antigen via the hypervariable region (antigen binding site)
- 4 chains
    - 2 identical heavy (H)
    - 2 identical light (L)
    - Each have a constant and a variable region
    - Heavy and light chains paired by disulphide bond
    - Heavy chains paired with each other by disulphide bonds
- 5 isotypes

IgG	IgD	IgA	IgE	IgM
• Most abundant • Monomeric • Major Ab of 2° immune response • Longest half-life (20 – 24 days) • Immune-experienced	• On B cell membrane • Extended hinge region • Function unknown	• Dimer • Predominant Ab in mucous secretions • Found on mucosal surfaces	• Pentamer held together by J chain • Major Ab of 1° immune response • Additional C region and amino acid tailpiece for multimerisation	• 2 additional C regions • Pentamer with 10 binding sites • On basophils and mast cells • Allergies, parasites, helminths

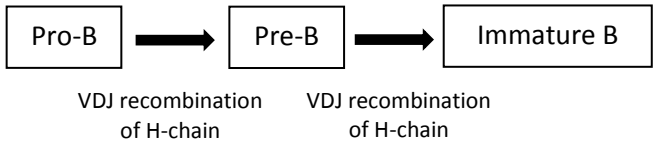
- Variable region for antigen binding
    - Site of somatic hypermutation
- Constant region for effector function, distinct distribution and access in body
    - Class switch recombination occurs on H_C region

B cell Development

- B cells are produced throughout life in bone marrow
- Originate from stem cells close to endosteal (inner) surface of bone
- Immature B cells only able to leave bone marrow if they express functional BCR on surface
- Exit via sinusoids (checkpoints) and migrate to periphery
- Surface Ab is antigen receptor
- Binding of antigen → CD4 T cell help → differentiation into memory cells and plasma cells
- B cell response to antigen changes with time (affinity and effector functions)
- VDJ recombination of heavy chain occurs in the Pro-B cell
- In Pre-B cell, heavy chains pair with 2 surrogate light chains and signalling chains (Igα and Igβ) to form pre-BCR
    - Since real light chains have not been formed yet
    - Surrogate light chains are the same in every B cell (not formed by rearrangement)
    - Pre-BCR then sends success signal into cell (V_H success signal)
        - Mutations that block this signal block development (e.g. Igα/Igβ, BLNK, Syk, Btk)
- In immature B cell, the real light chain pairs with existing heavy chain and signalling chains (Igα and Igβ) to form complete Ab (BCR)
    - BCR sends success signal sent into cell (V_L success signal)
- Immature B cells expressing BCR leave bone marrow and migrate to spleen
    - Naïve B cells express IgM and IgD on their surface
    - Naïve B cell populations found in also in lymph nodes, mucosal surfaces, gut
    - Await specific Ag and T cell help before further differentiation
- Mature B cells
    - Down-regulation of VDJ machinery
    - Express both IgM and IgD with same antigen specificity (identical variable regions)

Immune Response

- Affinity = binding strength
- Avidity = overall strength of Ab-Ag complex
- Primary immune response
    - Initial exposure to antigen
    - IgM secreted (low affinity, high avidity), followed by IgG/IgA (higher affinity)
    - Class switch recombination
        - Directed by signals from T cells and/or antigen
        - Ab specificity remains unchanged
- Secondary immune response (memory response)
    - Faster (same IgM response but faster CSR to IgG)
    - Greater magnitude (more Ab produced)
    - Higher affinity Ab than primary response due to Affinity Maturation by Somatic Hypermutation
- Lymphocyte activation
    - Antigen enters lymphoid organ and presented to T cell via APC
    - Antigen-specific B/T cells activated, T cells express CD40L, B cells express CD40
    - Activated B and T cells migrate towards each other and meet at T/B cell boundary
    - Co-stimulation occurs via ICOS (T cell) and ICOSL (B cell)
- Germinal centres
    - Site in 2° lymphoid tissue where mature B cells proliferate into memory and plasma cells
    - Proliferation, isotype switching, somatic hypermutation, affinity maturation, death and memory formation occur here
- Isotype switching/Class Switch Recombination
    - Changing constant region of antibody heavy chain (H_C) to change effector function
    - Directed by cytokine signals by T cells and CD40/CD40L interaction and/or antigen
    - Transcription of H chain gene starts from promoter upstream of V_H segment therefore exons encoding constant regions cannot be expressed until a V segment is placed upstream of them
        - Order: IgG3, IgG1, IgE, IgA1, IgA2, IgG2, IgG4, IgE, IgA2
    - Each constant region has a switch (S) region upstream
        - Rearrangement occurs between to S regions
    - Activated Induced Cytosine Deaminase (AID) enzyme introduces nicks into S region DNA, creating double stranded breaks in 2 S regions (now have S-S sequence)
        - Expressed only in activated B cells therefore in all germinal centres
    - Ends are joined and intervening DNA deleted
- Somatic hypermutation
    - Random introduction of random point mutations by AID into V gene segments of heavy and light chains in germinal centres B cells
    - May cause amino acid replacements, affecting antigen binding affinity (may increase or decrease)
    - Occurs during B cell proliferation, in dark zones of germinal centres
    - Occurs in hotspots of DNA (hypervariable complementary determining regions)
    - Not all B cells undergo this
- Affinity maturation
    - Selection of matured B cells with improved affinity → continue to proliferate
    - Low affinity B cells die
    - Tested on follicular dendritic cells

Mechanisms to generate B cell diversity of BCR	
Somatic Recombination	
Pairing of various heavy and light chains	Pre-BCR formed in Pre-B cell with surrogate light chain, final BCR formed in Immature B cell
Junctional diversity	Random nucleotide addition in H-chain by Tdt

Somatic Hypermutation and Affinity Maturation	Single point mutation via AID and selection of high affinity binding Ab

Immunoglobulin Genes

- 2 light chain loci (VJC)
  - λ on chromosome 22
  - κ on chromosome 2
- 1 heavy chain locus (VDJC) on chromosome 14
- Constant region
  - On each of the 3 loci
  - Distinct sequence for every isotype (IgG1-4, IgA1-2, IgM, IgE, IgD)
- Variable region
  - Heavy chain rearranged first, then one of the light chains
  - Other light chain rearranged during receptor editing prior to exiting bone marrow
  - 1,100,000 possible V_H/V_L combinations from 115 minigene segments
- Same processes and proteins used in T and B cell development
- **VDJ recombination** (occurs in Pro-B cell for H-chain)
  - Step 1: Site recognition and DNA cleavage
    - Recombination Signal Sequences (RSS) adjacent to each VDJ gene segment is recognised by RAG complex (RAG1, RAG2, HMG1) and cut, forming double stranded DNA breaks
    - Cut ends are stitched together (ligated) to form
      - Coding joint (hairpins) – V-J, V-DJ for H chains and V-J for L chains
      - Signal end joints – bit that is cut out by RAG complex (intervening DNA)
  - Step 2: Non-homologous end joining
    - DNA protein kinases bind to each DNA end and recruits Artemis, Ku70 and Ku80
    - Autophosphorylation of DNA protein kinases activates Artemis
    - Artemis is a nuclease with both endo- and exonuclease activity
      - Closes signal end to form signal joint (non-homologous end joining)
      - Opens coding hairpins
  - Step 3: Ligation
    - DNA protein kinases and XRCC4 (DNA ligase) align DNA ends and recruit Tdt enzyme (DNA polymerase)
    - Tdt adds random nucleotides to coding end hairpins in H-chain until complementary sequence is achieved
      - Creates more diversity
    - Exonucleases remove unpaired bases from coding ends
    - DNA polymerase λ and μ fill in blank complementary nucleotides
    - Coding ends are then ligated by DNA ligase IV
- Diversity is generated by
  - Random selection and rearrangement of VDJ minigene segments
  - Independent rearrangement at heavy and light chain loci
  - Random insertion of nucleotides by Tdt
- Mutations in rearrangement proteins (RAG, Artemis, etc.) can block development
- Tdt generally absent at light chain loci

Proteins involved in VDJ recombination	
RSS	Recombination Signal Sequence; a unique nucleotide sequence that is the RAG recognition sequence; is adjacent to each minigene segment (VDJ)
RAG1/2	Recombination Activating Genes are essential to Ab gene recombination; they provide the recognition and DNA cleavage activity
HMG1	High Mobility Group 1 protein; chromatin binding; structural protein also required for rearrangement but not unique to Ab gene rearrangement
Artemis/DNA protein kinases/Ku70/Ku80	Recognition and synapsis of the DNA ends
DNA ligase IV/XRCC4	X-ray Repair Complementing Chinese hamster Cells; Joining DNA ends

Tdt	Terminal Deoxynucleotidyl Transferase; unique to B cells; adds extra, random nucleotides to the broken ends at V-D-J junctions; does not work at light chain loci

Tolerance

- B cells are screened for autoreactivity at immature B cell stage (in bone marrow)
    - High affinity → apoptosis
    - Low affinity → anergic
- Receptor editing rearranges other light chain to escape autoreactivity (second chance)
    - Reactivation of VDJ rearrangement machinery

Primary Immunodeficiencies (PIDs)

- >350 rare genetic disorders
- Impaired ability to produce normal immune response
- Genetic in nature
- **Antibody deficiencies** (mostly) **–** humoral (B cell) immunity affected
    - X-linked agammaglobulinaemia (XLA)
    - Common Variable Immunodeficiency (CVID)
    - Hyper IgM Syndromes (HIGM)
- **Combined Immunodeficiencies –** humoral (B cell) and cellular (T cell) immunity affected
    - Severe Combined Immunodeficiency (SCID)
        - Defect in T cell differentiation resulting in impaired B cell function (due to no T cell help)
- Treatment
    - Replace entire immune system (e.g. in SCID, XLA) via
        - Haematopoietic stem cell transplantation (HSCT)
        - Gene therapy
    - Those with some antibody can undergo host response therefore are instead given intravenous antibody replacement (IVIG) or subcutaneous antibody replacement (SCIG)
        - E.g. HIGM, CVID

Severe Combined Immunodeficiency (SCID)

- E.g. the boy in the bubble (David Vetter)
- Lack of T cell differentiation
    - Therefore, no T cells
- Lack of B cells (due to no T cell help)
    - Therefore, little to no serum antibody of any isotype
- Recurrent viral, fungal and bacterial infections
- Diagnosis – newborn screening
    - Absence of signal joints is evidence of absence of B/T cell development
    - Quantification of TRECS (TCR excision circles) and KRECS (kappa-deleting recombination excision circles) by PCR used to diagnose SCID
    - No TRECS → No T cells
    - No KRECS → No B cells
- Treatment
    - Medications – antibiotics, antifungals, antivirals for infections
    - Avoiding exposure
    - Antibody supplementation (IVIG)
    - Enzyme replacement therapy – for ADA deficiency
    - Haematopoietic stem cell transplantation (HSCT) – most effective
        - Transplant from bone marrow, peripheral blood stem cells, umbilical cord stem cells
        - Produce a continuous supply of healthy lymphocytes

        - Immune suppression not required due to absence of T cells
    - Gene therapy – ongoing clinical trials

X-Linked Agammaglobulinaemia (XLA)

- Due to mutation in Bruton's Tyrosine Kinase (BTK)
    - Involved in the pre-BCR success signal pathway
    - Some forms result in residual Btk expression and signalling
        - Mild disease, later onset
    - Complete loss-of-function of Btk
        - Severe, early-onset
- No B cells
    - Therefore, little to no serum Ab of all isotypes
- Delay in presentation of disease
    - Maternal Abs offer protection in first months
- Recurrent infections
    - Greater risk of life-threatening bacterial pneumonias and neurological viral encephalitis
- Diagnosis
    - Test Ab levels
    - CD19+ B cell count
    - Btk sequencing
    - Gene-based testing: prenatal, Sanger sequencing, KRECS newborn screening
- Treatment
    - Intravenous Ab supplementation (IVIG)
    - Subcutaneous Ab supplementation (SCIG) – self-administered

Hyper IgM Syndromes (HIGM)

- X-linked CD40L deficiency (HIGM1)
    - Cellular, humoral and innate defects
    - Defective B cell differentiation, no germinal centres
    - No immune memory
- Pathogenesis
    - Class switch recombination (CSR) defects → T cell defects
    - Somatic hypermutation defects → lack of high affinity Abs, T cell defects
- Decreased IgG, normal-high IgM
- Variable symptoms and severity
- Extremely rare
- Treatment
    - IVIG, SCIG
    - Bone marrow transplant, gene therapy for XL-HIGM

Common Variable Immunodeficiency (CVID)

- Largest class of PIDs
- Most common PID in Australia and worldwide
- B cells fail to differentiate
- Diagnosis
    - Hypogammaglobulinaemia (reduced IgG)
    - Recurrent bacterial infections
    - No Abs even when given vaccine
- Treatment
    - Monthly IVIG or SCIG infusions
        - Effective for infectious-only phenotype
        - Many patients have multiple complications