Immunity Mediated by B cells and Antibodies
Chapter 9 Parham

Rob Roovers
26th of May 2010
Agenda

- Antibody production by B lymphocytes
  - Surface immunoglobulin and co-receptor
  - Activation by CD4 T cells
  - IgM secretion plasma cells
  - Hypermutation and isotype switch
  - Differentiation of memory cells

- Effector functions
  - Protection internal tissues by IgM, IgA and IgG
  - Dimeric IgA protects mucosal tissues
  - High affinity antibodies for neutralization of viruses and toxins
  - Complement activation by IgM and IgG
  - Subclasses of IgG and their characteristics
  - Role of Fc receptors in clearance of pathogens
Function of antibodies

- Not toxic or destructive to pathogens
  - bind antigen
  - neutralise pathogen (prevent entry)
  - opsonise antigen: phagocytosis
  - activate complement: phagocytosis
- Affinity and effectivity increases during immune response (somatic hypermutation)
- Some bacterial antigens induce T-cell independent antibody responses (IgM)
- Function depends on isotype of antibody
B-cell activation requires cross-linking of the BCR

- Surface IgM/IgD bind antigen: clustering
- Signal is transduced via Igα and Igβ

Figure 9.1 The Immune System, 3ed. (© Garland Science 2009)
B-cell activation requires cross-linking of the BCR

- Cytoplasmic associated protein tyrosine kinases
- ITAM bind Blk, Fyn and Lyn, becomes phosphorylated
- Syk binds to P-ITAM Igβ and transduces signal
Full B-cell activation requires an additional signal (e.g. via the B-cell co-receptor)

- CR2 recognises iC3b and C3b breakdown products
- CD19 signalling component; CD81 unknown function
Generation of iC3b by CR1

- C3b deposited on pathogen is recognised by CR1
- Cleavage by factor 1 yields iC3b and C3d
- CR2 recognises C3d

Figure 9.4 The Immune System, 3ed. (© Garland Science 2009)
Clustering of BCR and co-receptor fully activates the B-cell

- Clustering of BCR and B-cell co-receptor

- Igα-associated Lyn in proximity of CD19: phosphorylation of CD19

- Simultaneous ligation of BCR and co-receptor synergise and thereby increase sensitivity to antigen

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Figure 9.5 The Immune System, 3ed. (© Garland Science 2009)
T-cell–independent antigens

- Thymus independent (TI) antigens: patients who lack a thymus mount antibody responses to certain antigens
  - only/predominantly IgM antibodies; no class-switch (no cytokines from T helper cell)

- Two types of antigens: TI-1 and TI-2; need for alternative co-stimulation
  - TI-1: second signal from TLR (e.g. LPS)
  - TI-2: multivalent Ag, extensive cross-linking of BCR, no second signal necessary

- No induction of immunological memory
T-cell–dependent antigens

- Thymus dependent antigens: in secondary lymphoid organs, Ag, B-cell and Th cell are brought together

- DC’s bring antigens from infected tissue to the draining lymph node (‘spread’ the infection)
Co-operation of B- and T-cells; the formation of cognate pairs

- BCR: 2 functions (bind Ag and internalise/present Ag)
- In Ag-specific B-cell, the BCR internalises with Ag and causes presentation in MHC II
- Ag-specific, activated T cells ‘scan’ passing B-cells with their TCR in the T-cell zone
- T-cell expresses CD40L, ligation to CD40 and the formation of a cognate pair

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The immunological synapse

- Cognate pair: Increased adhesion (LFA1-ICAM-1)

- Re-orientation of secretory apparatus

- Release of cytokines (notably IL-4)
The primary focus

• Cognate pair: expansion of both B- and T-cell

• Movement out of T-cell zone to medullary cord

• B-lymphoblasts produce IgM that is delivered to the blood

• Terminal differentiation of B-cells to plasma cells is mediated by BLIMP-1 (B-lymphocyte-induced maturation protein-1)
Follicular dendritic cells

- Not hematopoietic cells, but stromal cells
- Essential for B-cell survival, maturation and isotype switching
- Important function in creating a deposit of Ag (by means of CR1 and CR2 expression)

“Tccosomes”
Somatic hyper-mutation and isotype switching

- Cognate pairs move from the primary focus to the B-cell zone (primary follicles)
- FDC and T-cells make the B-cells undergo proliferation and somatic hypermutation
- IL-6/15/8D6 (FDC) $\rightarrow$ proliferation; CD40L (T-cell) $\rightarrow$ activation induced cytidine deaminase (isotype switching)
The germinal center

- Rapidly dividing B- and T-cells: recombination of the Ig genes (risk: cancer!!)
- ‘Dark zone’: densely packed centroblasts
- light zone: centrocytes, FDC and T-cells

Mantle zone: naive B-cells

Figure 9.14 The Immune System, 3ed. (© Garland Science 2009)
Selection in germinal centers drives affinity maturation of antibodies

• Centrocytes die quickly by apoptosis if not positively selected (by both BCR and CD40)

• Ag is ‘picked up’ from the FDC: competition for Ag drives up the affinity of the selected antibody

• Ag is processed, presented to Th cells

• Ligation of TCR and CD40L induces expression of Bcl-\textsubscript{XL} in B-cell and leads to survival of B-cell

• Self-reactive B-cells are made anergic
Selection in germinal centers drives affinity maturation of antibodies

Figure 9.15 The Immune System, 3ed. (© Garland Science 2009)
Affinity maturation and isotype switching in germinal center B-cells

• The isotype to which the antibody is switched depends on the cytokine profile secreted by the T-cell

• CD40-CD40L interaction is vital for switching (hyper-IgM syndrome in people who lack CD40L: no germinal centers)
Cytokines determine differentiation of selected centrocytes to either plasma or memory B-cell

- **IL-10** → plasma cells
- **IL-4** → memory B-cells
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IgM, IgG and monomeric IgA are Ab formats present in blood

- IgM
  - first antibody produced
  - secreted as pentamer, avid binding to microorganisms and multivalent Ags
  - activates complement: coating pathogen with C3b enables uptake and destruction by phagocytes
  - large, not efficiently penetrating infected tissues

- IgG
  - better affinity due to somatic hypermutations
  - bivalent and smaller resulting in better tissue penetration
  - actively delivered into tissues via FcRn

- Circulating IgM, IgG and IgA prevent blood-borne infections
FcRn (Brambell receptor) mediated IgG delivery

- Endothelial cells ingest small amounts of blood containing IgG (pinocytose)
- Plasma proteins degraded in endocytic vesicle, but IgG protected by binding to FcRn
- IgG binds via Fc to FcRn at low pH and the endocytic vesicle and is transported from apical (blood) to basolateral side; dissociates at basic pH from FcRn
- FcRn similar structure as MHC I
- by recycling via FcRn IgG protected against proteolysis in blood (long serum half life)
Dimeric IgA is transported across mucosal epithelia

- Dimeric IgA protects surfaces of mucosal epithelia that communicate with external environment
- Dimeric IgA binds covalently via J chain (linking 2 IgAs) to polymeric Ig receptor or pIgR
- Receptor mediated endocytosis and subsequent transport of vesicles from basolateral to apical side
- Protease cleaves within receptor and releases IgA with part of receptor (called secretory component or SC)
- Carbohydrates within SC bind to mucins on mucosal surface; exposed IgA can bind microorganisms and thereby prevent colonization and facilitate expulsion

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IgE is quickly bound by FcεR1 and ‘coates’ mast cells, scanning for antigen

• IgE made in smaller quantities than IgG/IgA

• Once synthesised, IgE is quickly and tightly bound by FcεR1 on mast cells and baso- / eosinophils

• Once recognising it’s antigen, the mast cell releases mediators that trigger smc contraction

• Works in the connective tissues (bound to mast cells) to cause physical ejection of the pathogen (sneezing, coughing, vomiting)
Mother’s antibodies protect child before and after birth

- **Before birth:**
  - IgG transported from mother’s circulation via placenta
  - mediated via FcRn
  - high level IgG in child’s blood obtained from mother protecting against pathogens

- **After birth:**
  - dimeric IgA via breast-feeding transferred to baby’s gut
  - mediated via pIgR
  - protects against micro-organisms infecting mucosal surfaces

- After 6 months maternally derived IgG catabolized and consumption of breast milk stops
- Infant’s own immune system develops and starts to produce Ab
- IgG levels lowest in infants of 3 – 12 months, most susceptible to infections
Neutralization of viruses and bacteria by high affinity Abs

- First step in infection of bacteria and viruses is attachment of pathogen’s outer surface protein to cellular receptor on cells of host.
- High affinity Ab’s directed against epitope of pathogen’s protein responsible for binding to receptor will block interaction and prevent infection.
- Hemagglutinin is receptor binding protein on envelope of Influenza virus; interacts with oligosaccharides of cell surface glycoproteins.
- Adhesins (f.i. protein F of *Streptococcus pyogenes*) are bacterial receptor binding proteins interacting with host’s fibronectin.

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Neutralization bacterial toxins and animal venoms by IgG and IgA

- Bacterial toxins bind to receptors causing disease by disrupting normal functions of cells
- Neutralizing Abs bind to chain or part of toxin interacting with receptor
- High affinity IgG for neutralization in tissues, high affinity IgA dimer at mucosal surfaces
- Same principle for venoms from snakes and other animals; transfer of protective Abs raised in animals (f.i. horse)
  => passive immunization

<table>
<thead>
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<th>Disease</th>
<th>Organism</th>
<th>Toxin</th>
<th>Effects in vivo</th>
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<tr>
<td>Tetanus</td>
<td>Clostridium tetani</td>
<td>Tetanus toxin</td>
<td>Blocks inhibitory neuron action, leading to chronic muscle contraction</td>
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<tr>
<td>Diphtheria</td>
<td>Corynebacterium diphtheriae</td>
<td>Diphtheria toxin</td>
<td>Inhibits protein synthesis, leading to epithelial cell damage and myocarditis</td>
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<tr>
<td>Gas gangrene</td>
<td>Clostridium perfringens</td>
<td>Clostridial-α toxin</td>
<td>Phospholipase activation, leading to cell death</td>
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<td>Cholera</td>
<td>Vibrio cholerae</td>
<td>Cholera toxin</td>
<td>Activates adenylate cyclase, elevates cAMP in cells, leading to changes in intestinal epithelial cells that cause loss of water and electrolytes</td>
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<tr>
<td>Anthrax</td>
<td>Bacillus anthracis</td>
<td>Anthrax toxic complex</td>
<td>Increases vascular permeability, leading to edema, hemorrhage, and circulatory collapse</td>
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<tr>
<td>Botulism</td>
<td>Clostridium botulinum</td>
<td>Botulinum toxin</td>
<td>Blocks release of acetylcholine, leading to paralysis</td>
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<tr>
<td>Whooping cough</td>
<td>Bordetella pertussis</td>
<td>Pertussis toxin Tracheal cytotoxin</td>
<td>ADP-ribosylation of G proteins, leading to lymphocytosis Inhibits ciliary movement and causes epithelial cell loss</td>
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<tr>
<td>Scarlet fever</td>
<td>Streptococcus pyogenes</td>
<td>Erythrogenic toxin Leukocidin Streptolysins</td>
<td>Causes vasodilation, leading to scarlet fever rash Kill phagocytes, enabling bacteria to survive</td>
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<tr>
<td>Food poisoning</td>
<td>Staphylococcus aureus</td>
<td>Staphylococcal enterotoxin</td>
<td>Acts on intestinal neurons to induce vomiting. Also a potent T-cell mitogen (SE superantigen)</td>
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<tr>
<td>Toxic-shock syndrome</td>
<td>Staphylococcus aureus</td>
<td>Toxic-shock syndrome toxin</td>
<td>Causes hypotension and skin loss. Also a potent T-cell mitogen (TSST-1 superantigen)</td>
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</table>

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Complement activation by IgM

- On binding to surface of pathogen pentameric IgM, adopts a ‘staple’ conformation

Initiation of the classical pathway of complement by IgM binding to antigen on pathogen surface

- Binding site on Fc for C1q accessible; avid binding to C1q (5 sites within IgM, C1q has 6 IgM binding sites)
Complement activation by IgM

- Binding of C1 to IgM results in activation of serine proteases C1r and C1s (part of C1), latter cleaves and activates C2 and C4 of classical complement pathway; different C4 alleles exist with complementary functions, lack of some leads to diseases
- Formed product C4b binds covalently to pathogen’s surface and interacts with C2a; complex binds classical C3 convertase, which is cleaved in C3a and C3b, latter gets covalently bound to surface of pathogen
- C3b binds Bb to form the alternative convertase C3bBb resulting in deposition of many more C3b fragments on surface and phagocytosis
At least two IgGs needed for activation of complement

- IgG3 can activate complement, IgG1 to lower degree followed by IgG2
- At least two molecules have to bind to one IgG molecule
- Activation dependent on amount and density of IgG in complex with antigen (should be in close proximity)
- High affinity allows IgG to bind to soluble multivalent antigens

- Immune complexes with soluble antigens activates complement leading to clearance via phagocytosis
- Erythrocytes transport complexes by interacting via CR1 receptor to C3b deposited on antigen
- Liver main organ for clearance of complexes; if it can’t process large amounts of immune complexes, then kidney comes into play
Characteristics of IgG

- Conformational flexibility of hinge allows avid binding of 2 Fab arms and independent binding of Fc receptor to Fc

- Hinge is linear structure susceptible for cleavage by proteases
Characteristics of IgG subclasses

- Four subclasses discriminated numbered according to presence in serum

- IgG1 intermediate in flexibility, protease sensitivity and complement activation
- IgG2 has hinge with 4 disulfide bonds reducing flexibility, protease sensitivity and complement activation; binds repetitive carbohydrates on surface microorganisms (T1 Ags)
- IgG3 contains long hinge with best flexibility => best in avid binding and interaction with complement C1, but sensitive for proteases
- IgG4: no effector functions, only neutralization
  => exchange of heavy chain with other IgG4 in blood leading to monovalent binding
  => binding to allergens competes with IgE and reduces severity of allergic reaction

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## Properties of IgG subclasses

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<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
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<tr>
<td><strong>Proportion of total IgG (%)</strong></td>
<td>45–75</td>
<td>16–48</td>
<td>2–8</td>
<td>1–12</td>
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<tr>
<td><strong>Length of heavy-chain hinge (amino acids)</strong></td>
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<td>12</td>
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<td><strong>Number of disulfide bonds in the hinge</strong></td>
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<td><strong>Half-life in serum (days)</strong></td>
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<td><strong>Capacity to bind C1q and activate complement</strong></td>
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High affinity receptor FcγRI activates hematopoietic cells

- Associated γ dimer with ITAM motifs responsible for signalling
- Hierarchy of binding: IgG3>IgG1>IgG4>>>IgG2
- Circulating IgG3 and IgG1 bind to receptor, but X-linking in immuno-complex necessary for signalling

• FcγRI expressed on myeloid cells
  => constitutively on monocytes, macrophages and DCs
  => induced on neutrophils/eosinophils

• Extracellular domain exists of 3 Ig domains involved in interaction with Fc (hinge – CH2)

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Low affinity receptors FcγRII and FcγRIII

- Cross-linking via antigen necessary for binding and signalling
- FcγRIIA activating, i.e. promoting uptake and destruction by myeloid cells; has ITAM domain
- FcγRIIB2 inhibitory, present on same cells as RIIA; controls inflammatory responses
- FcγRIIB1 inhibitory receptor on mast and B cells; negative regulator
- FcγRIIB1 and FcγRIIB2 contain ITIMs (Immunoreceptor tyrosin-based inhibitory motifs)
- FcγRIIA present in 2 main allotypes:
  => H131 binds IgG2 as well as IgG1
  => R131 ineffective in binding IgG2
- Individuals homozygous for R131 allotype less effective in clearance of IgG2 coated bacteria, increased risk of fulminant meningococcal disease and septic shock on infection with *Neisseria meningitidis*

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Low affinity FcγRIII responsible for ADCC

- FcγRIII expressed on NK cells and occurs in two forms:
  - FcγRIIIa associated with signaling dimer γ chain
  - FcγRIIIb attached to outer membrane via GPI, not associated with γ chain (but is activating)
- Expressed on NK cells that can kill human cells coated with IgG1 or IgG3 directed against cell surface targets
  - Rituximab against B cell marker CD20
  - Viral glycoproteins expressed on surface infected cells
- Two allotypes of FcγRIIIa, one of which is giving better ADCC (efficacy Rituximab better in higher affinity allotype)

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Receptors for IgE and IgA

- FcεRI expressed on mast cells, basophils and activated eosinophils
- High affinity of Fc for receptor results in coating of IgE on cells (many specificities per cell)
- Mast cells release histamin and other inflammatory mediators from granules upon antigen induced by cross-linking of IgE
- Mediators increase permeability of vessels enabling cells and molecules of immune system and to move into tissues and outflow of fluids
- IgE directed against multicellular parasites and applying different strategy then used for microbes
- Violent muscular contraction and outflow of fluids physically removes parasite pathogens
- FcαRI comparable to FcγRI, IgG and IgA similar
- Gene for FcαRI encoded by chromosome 19, whereas all FcγR genes are on chromosome 1

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# Summary Fc receptors

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<tr>
<th>Ligand</th>
<th>FcγRI</th>
<th>FcγRIIA</th>
<th>FcγRIIB</th>
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