Adaptive Immunity: Humoral Immune Responses

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Synopsis: In this lecture we will review the different mechanisms which constitute the humoral immune response, and examine the antibody molecules which are involved.

Outcomes: You should be able to describe the antibody molecules and their functions; the process by which antibody repertoire diversity is generated; how and where B cells are activated to produce antibody; the T-dependent and T-independent antigens; the various mechanisms by which B cells and antibodies protect the host from disease.
Antibody Protection of the Host

Neutralization:
Antibody prevents bacterial adherence

Opsonization:
Antibody promotes phagocytosis

Complement activation:
Antibody activates complement, which enhances opsonization and lyses some bacteria
Features of primary and secondary antibody responses

A diagram illustrating the features of primary and secondary antibody responses. The primary antibody response involves activated B cells leading to the production of IgM and IgG antibodies in peripheral lymphoid tissues. In contrast, the secondary antibody response shows a higher level of antibody production, with plasma cells in bone marrow and memory B cells.
Antibodies

• Antibodies are the secreted form of the B cell receptor
• Y-shaped molecules consisting of 3 equal-sized portions connected by a flexible tether
• Each immunoglobulin molecule is made up of two heavy chains and two light chains joined by disulphide bonds so that each heavy chain is linked to a light chain and two heavy chains are linked together

• Two types of light chains, kappa (κ) and lambda (λ) are found in antibodies
  • κ: λ = 2:1 in humans
  • An antibody has either kappa or lambda light chains, never one of each. No functional differences ever identified
Antibody classes

• The class, and thus the effector function, of an antibody is determined by its heavy chain
• There are five main heavy-chain classes or isotypes, some of which have several subtypes:
  – IgM (μ)
  – IgD (δ)
  – IgG (γ) : IgG1, IgG2, IgG3, IgG4
  – IgA (α)
  – IgE (ε)
IgM and IgA molecules can form multimers

IgM and IgA are usually synthesized as multimers in association with an additional polypeptide chain, the J chain.
Primary antibody repertoire is diversified by different processes

The initial antibody repertoire of B cells developing in bone marrow is derived by recombination of the Variable, Diversity, and Joining genes. These somatic mutations take place without B cell encountering antigen.

Secondary, diversifying mutation takes place in activated B cells after antigen encounter:
- somatic hypermutation
- class switching
The frequency of Ig gene mutations occurring in germinal centres is about 1 in $10^3$ base pairs/cell division.

This extensive mutation results in generation of different B cell clones with range of affinities for antigen that initiated the response.

**Affinity maturation** is the process by which the affinity of antibodies produced in response to a protein antigen increases with prolonged or repeated exposure.
Antigen-stimulated B cells may differentiate into IgM-secreting cells, or, under the influence of CD40L and cytokines (=T cell help) differentiate into cells that produce different Ig heavy chain isotypes.
Humoral immune response is mediated by antibodies secreted by plasma cells

Antigen that binds to the BCR:
• sends a signal into the B cell
• is internalized and processed into peptides that activate effector CD4+ T cells

Signals from the bound antigen and from the T cell induce the B cell to proliferate and differentiate into plasma cells secreting antibody

Antibodies protect the host by Neutralization, Opsonization, and Complement activation
Activation of naïve B cells requires accessory signals

- Antibody responses to protein antigens require antigen-specific CD4+ T cell help
- These protein antigens are unable to induce antibodies in humans who lack T cells, therefore they are called thymus-dependent (TD) antigens
- To receive T cell help, the B cell must be displaying antigen on its surface in a form a T cell can recognize: in the context of an MHCII molecule, i.e., the B cell has processed the antigen it has internalized
- The T cell must have been previously activated from a naïve to an effector state: it was activated by a dendritic cell presenting the correct antigen, in the lymph node
Thymus-dependent (TD) antigen

• First signal required for B cell activation is delivered through its antigen receptor
• Second signal is delivered by a helper T cell that recognizes degraded fragments of antigen as peptides bound to MHC Class II molecules on the B cell surface
• Interaction between the CD40 ligand (CD40L or CD154) on the T cell and CD40 on the B cell is essential
Thymus-independent (TI) antigens

• Some microbial constituents, such as bacterial polysaccharides, can induce antibody production in the absence of helper T cells
• These antigens are known as thymus independent antigens because they can induce antibody responses in individuals with no T cells
• TI antibody responses provide some protection against extracellular bacteria
Thymus-independent (TI) antigens

• First signal required for B cell activation is delivered through its antigen receptor
• Second signal can be delivered along with the antigen, via TLRs that recognize antigen-associated TLR ligands such as LPS or bacterial DNA
• B cells and helper T cells must recognize epitopes of the same molecular complex in order to interact

• Here, an epitope on a viral coat protein is recognized by surface Ig and the virus is internalized and degraded

• Peptides derived from the viral proteins are returned to the B cell surface bound to MHC Class II molecules

• These MHCII:vPeptide complexes are recognized by helper CD4+ T cells, which help to activate the B cells to produce antibody against the coat protein
Linked recognition and conjugate vaccines

- Infants make weak antibody responses to TI antigens
- Adults make strong protective responses to Haemophilus influenzae type B, a bacteria that causes meningitis
- To make an effective vaccine that will protect children, the H.influenzae polysaccharide is linked chemically to tetanus toxoid, a protein against which infants are successfully vaccinated
- B cells that bind the polysaccharide component of the vaccine are activated by helper T cells specific for tetanus toxoid antigen, generated previously

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B cells encounter T cell in lymph nodes

• The chance of an encounter between a T cell and a B cell that recognize the same antigen is between $1 \text{ in } 10^8$ and $1 \text{ in } 10^{12}$
• In addition T and B cells occupy different regions of the lymph node: the T cell areas and the primary lymphoid follicles (or B cell areas)
• Linked recognition therefore requires precise regulation of the migration of activated B and T cells into specific locations within the lymphoid tissues
• Naïve B cells are shown antigen by follicular dendritic cells (FDC) and specialized macrophages
• B cells which encounter their antigen move towards the T cell area. If they do not interact within 24 hours they die
• Activated cells migrate to form a primary focus and here, differentiate into plasmablasts
Antigen-binding B cells meet T cells at border between T-cell area and B cell follicle in secondary lymphoid tissue

Plasmablasts are cells that have begun to secrete antibody but are still dividing. After a few days, plasmablasts in primary focus stop dividing and either die, or undergo terminal differentiation into plasma cells. Plasma cells that remain in lymphoid organ are shortlived; those that migrate to bone marrow continue antibody production there.
Immunoglobulin classes are selectively distributed in the body

- IgG and IgM predominate in blood
- IgG and monomeric IgA are major antibodies in extracellular fluid within the body
- Dimeric IgA predominates in secretions across epithelia, including breast milk
- Foetus receives IgG from the mother by transplacental transport
- IgE is found mainly associated with mast cells just beneath epithelial surfaces particularly in respiratory tract, gastrointestinal tract and skin
Distribution and function of immunoglobulin classes: IgA

Principal sites of IgA synthesis: gut, respiratory epithelium, lactating breast, other exocrine glands such as salivary and tear glands

Primary functional role of IgA antibodies is to protect epithelial surfaces from infectious agents.

Also thought to regulate gut microbiota
High affinity IgG and IgA can neutralize bacterial toxins

- Neutralization of toxins by IgG antibodies protects cells from their damaging action
- The damaging effects of many bacteria are due to the toxins they produce
- Antibodies that inhibit toxin binding can prevent, or neutralize, damaging effects
Viral infection of cells can be blocked by neutralizing antibodies

- A virus must first introduce its genes into a cell in order to replicate
- First step in entry is usually binding to a receptor on cell surface
- For enveloped viruses entry requires fusion of the viral envelope and cell membrane – can occur on cell surface or within endosomes
- Antibodies bound to viral surface proteins neutralize the virus, inhibiting either the initial binding, or its subsequent entry
Antibodies can prevent attachment of bacteria to cell surface

• Many bacterial infections require an interaction between the bacterium and a cell surface receptor, particularly so for infections of mucosal surfaces
  • Attachment process involves very specific molecular interactions between bacterial adhesins and their receptors on host cells
  • Antibodies against bacterial adhesins can block such infections
Fc receptors facilitate phagocytosis of antibody-bound cells

- Fc receptors are a family of cell-surface molecules that bind the Fc portion of immunoglobulins.
- Each member of the family recognizes one or a few closely related heavy-chain isotypes.
- Isotype therefore determines which types of cells will be engaged in a given response.
- Most prominent function of Fc receptors (FcR) is activation of accessory cells to attack pathogens.
Antibody dependent cellular cytotoxicity

- Antibody coated target cells can be killed by antibody-dependent cellular cytotoxicity (ADCC)
- Natural killer cells have FcγRIII (CD16) on their cell surface
- When NK cells encounter cells coated with IgG, they rapidly kill the target cell
IgE cross-linking on mast cells leads to rapid release of inflammatory mediators

- Mast cells are found in connective tissue
- Their secretory granules contain many inflammatory mediators
- They bind stably to monomeric IgE antibodies through FcεRI
- Antigen cross-linking of bound IgE triggers rapid degranulation, releasing inflammatory mediators into surrounding tissue
- These molecules recruit cells and proteins to site of infection
- Mast cells also triggered during allergic reactions when allergens bind to IgE on mast cells
IgG and IgM activate the Classical Complement pathway.

- Pentameric IgM molecules bind to antigens on the bacterial surface and adopt the ‘staple’ form.
  - ‘planar’ form of IgM
  - ‘staple’ form of IgM

- IgG molecules bind to antigens on the bacterial surface.

- C1q binds to one bound IgM molecule.

- C1q binds to at least two IgG molecules.

- Binding of C1q to Ig activates C1r, which cleaves and activates the serine protease C1s.

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Immune complexes are cleared from the circulation in the spleen

- CR1 on the surface of erythrocytes has an important role in clearance of immune complexes from the circulation

- Immune complexes bind to CR1 on erythrocytes, which transport them to the liver and spleen where they are removed by macrophages expressing receptors both for Fc and bound complement components