Chapter 4. Antigens

Terminology:

Antigen: Substances that can be recognized by the surface antibody (B cells) or by the TCR when associated with MHC molecules

Immunogenicity VS Antigenicity:

Immunogenicity – ability to induce an antibody and/or cell-mediated immune response

<u>Antigenicity</u> – ability to combine with the final products of the response (antibodies and/or T cell receptor)

NOTE: Most immunogenic molecules are also antigenic

<u>Hapten</u> - a small molecule that is <u>antigenic</u> but not (by itself) immunogenic.

Antibodies can be made to haptens only after the hapten is covalently conjugated to a large protein "carrier".

Figure 5



Factors that influence immunogenicity:

- Foreign-ness non-self (far apart evolutionary or phylogenetically)
- <u>Type of molecule</u> (chemical nature) protein > polysaccharide > lipid > nucleic acid
- Molecular Size >10,000 Daltons are more immunogenic
- -<u>Composition</u> heterogeneity increases immunogenicity. - 4ry > 3ry > 2ry > 1ry structure
- -Degradability protein antigens must be degraded (phagocytosis) in order to be presented to helper T cells.

Physical Form - Denatured > Native



Additional factors that influence the immune response:

- Genetics of the recipient (genotype MHC)
- Dosage of the antigen (optimal dose tolerance)
- Number of doses of the antigen (boosters)
- Route of administration of the antigen
 - intravenous (spleen)
 - subcutaneous (lymph nodes)
 intraperitoneal (lymph nodes)
 - oral (mucosal GALT)
 - orai (mucosai GALT)
 - inhaled (mucosal BALT))
- Use of adjuvant

Adjuvant: a substance that, when mixed with an antigen and injected with it, serves to enhance the immune response to the antigen.

Possible mechanisms of action of adjuvants:

- <u>Prolong the persistence of the antigen</u>, thus giving the immune system more time to respond
- Increase the "size" of the antigen by causing aggregation,
- Stimulate lymphocyte proliferation and/or activation
- Stimulate a <u>local inflammatory response</u>, thus recruiting cells to the site of the antigen (GRANULOMA)
- Enhance co-stimulatory signals

Commonly used adjuvants: (Table 3.3)

- Alum aluminum potassium sulfate precipitates the antigen, resulting in increased persistence of the antigen. Increases "size" of antigen → ↑ phagocytosis.
- Incomplete Freund's adjuvant mineral oil-based increases persistence of the antigen, mild granuloma.
- Complete Freund's Adjuvant mineral oil-based adjuvant containing dead *Mycobacterium* - increases persistence of the antigen, stimulates a chronic inflammatory response (granuloma), and co-stimulatory signals. Activates macrophages and DCs.
- Bacterial Lipopolysaccharides stimulate nonspecific lymphocyte activation and proliferation, and co-stimulatory signals.

Epitope or Antigenic Determinant - the region of an antigen that binds to a T cell receptor or a B cell receptor (antibody).

- Since an epitope is the part of the antigen that binds to the B cell or T cell receptor, it is the part that determines the antigenicity of the antigen - thus the term "antigenic determinant".
- -T and B cells recognize different epitopes on an antigen



Properties of B cell epitopes:

- Usually dependent on the native, tertiary conformation of the antigen (PROTEIN FOLDING)

- Must be accessible tend to be on the "surface" of the antigen (hydrophilic)
- May be made of sequential or non-sequential amino acid sequences (epitopes made up of non-sequential amino acid sequences are called "conformational epitopes").
- Binds to soluble antigen, No MHC molecule requirement
- Large antigens contain multiple, overlapping B cell epitopes.



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"B-lymphocytes have slg molecules on their surface that recognize epitopes directly on antigens. Different B-lymphocytes are programmed to produce different molecules of slg, each specific for a unique epitope."

animation and pictures from http://www.cat.cc.md.us/courses/bio141/lecguide/unit3/epsig.html

Properties of B cell epitopes (Table 3-4)

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Properties of T cell epitopes:

- Involves a tertiary complex: T cell receptor, antigen, and MHC molecule
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Properties of T cell epitopes:

- Involves a tertiary complex: T cell receptor, antigen, and MHC molecule
- Internal linear peptides (hydrophobic) produced by processing and bound to MHC molecules
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Properties of T cell epitopes:

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Basic Antibody Structure

- Multiple myeloma = cancerous plasma cells
- Monomer = 150,000



Summary

- Molecule consists of Constant and Variable regions for both Light and Heavy chains (CH, VH, CL, VL)
- Ig molecule made of domains = Ig fold
- Domains ~ 110 aa
- Each antigen-binding site is made up of the N-terminal domain of the heavy and the light chains
- IgM and IgE possess 4 CH domains (CH1-CH4). Hinge region is missing.
- IgG, IgA and IgD have 3 CH domains (CH1-CH3).
- Hypervariable regions in the variable regions of both H and L chains.







- A simulated antigenbinding site showing how the CDRs form points of contact with the antigen.

RECAP:

- Antibodies are comprised of repeating 110 aa units referred to as domains or Ig folds.
- The C-terminal domains are constant from antibody to antibody (within a class).
- The constant region domains are responsible for all functions of antibody other than antigen binding (opsonization, ADCC, complement activation) → Biological Function!
- The N-terminal domains are variable from antibody to antibody and are referred to as "variable domains".
- The variable domains contain 3 hypervariable regions the CDRs.
- The CDRs of the V domains in both H and L chains make up the antigen-binding site.

Antibody-Mediated Effector Functions

- Binding to Antigen \rightarrow endocytosis
- OPSONIZATION: FcR in macrophages and neutrophils (C3b) (IgG1, IgG3)
- ADCC NK cells (and other cells) trough FcR
- CROSSING EPITHELIAL LAYERS IgA (but also IgM)
- CROSSING PLACENTA- IgG (IgG1, IgG3, IgG4)
- COMPLEMENT ACTIVATION: IgG (IgG3) and IgM























Antigenic Determinants on Immunoglobulins

- Abs are glycoproteins and themselves very immunogenic
- Epitopes on immunoglobulins are divided into:
 - ISOTYPIC
 - ALLOTYPIC
 - IDIOTYPIC

















Monoclonal Antibodies

- Kohler & Milstein 1975
- Fusion of normal, activated B cell and plasmacytoma (cancerous plasma cell)



Plasmacytoma VS B cell

• Plasmacytoma:

- Cancerous plasma cell (Immortal)
- Does not secrete Abs
- Lacks HGPRT
- Normal spleen B cell
 - Limited life span
 - Secretes Abs
 - Possess HGPRT
 - Hybrid: immortal, secrete Ab, hypoxanthine (HGPRT)



Applications

- Diagnosis
- Therapeutics

RECAP - Sequence variation in antibodies:

- 1. Different light changes no significant functional effect
- 2. Different heavy chains very significant functional effect - isotypic variation
- 3. Allelic variation between individuals no large functional effect <u>allotypic variation</u>
- 4. Variation in the antigen-binding site idiotypic variation