Chapter 3. Antigens

Terminology:

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

Immunogenicity VS Antigenicity:

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

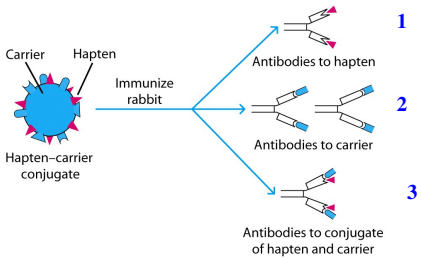
Antigenicity – ability to combine with the final products of the response (antibodies and/or T cell receptor)

NOTE: Most immunogenic molecules are also antigenic

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

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

Figure 5.1



Injection with:	Antibodies formed:
Hapten (DNP)	None
Protein carrier (BSA)	Anti–BSA
Hapten–carrier conjugate (DNP-BSA)	Anti–DNP (major) Anti–BSA (minor) Anti–DNP/BSA (minor)

Factors that influence immunogenicity:

- **Foreign-ness** – non-self (far apart evolutionary)
- **Type of molecule** (chemical nature) - protein > polysaccharide > lipid > nucleic acid
- **Size** - larger molecules tend to be more immunogenic
- **Composition** - 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

TABLE 3-1 MOLECULAR WEIGHT OF SOME COMMON EXPERIMENTAL ANTIGENS USED IN IMMUNOLOGY

Antigen	Approximate molecular mass (Da)
Bovine gamma globulin (BGG)	150,000
Bovine serum albumin (BSA)	69,000
Flagellin (monomer)	40,000
Hen egg-white lysozyme (HEL)	15,000
Keyhole limpet hemocyanin (KLH)	>2,000,000
Ovalbumin (OVA)	44,000
Sperm whale myoglobin (SWM)	17,000
Tetanus toxoid (TT)	150,000

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)
  - inhaled (mucosal)
- 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:

- Prolong the persistence of the antigen, 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 local inflammatory response, 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 and induces mild granuloma.

Incomplete Freund's adjuvant - mineral oil-based - increases persistence of the antigen, mild granuloma, and induces co-stimulatory signals

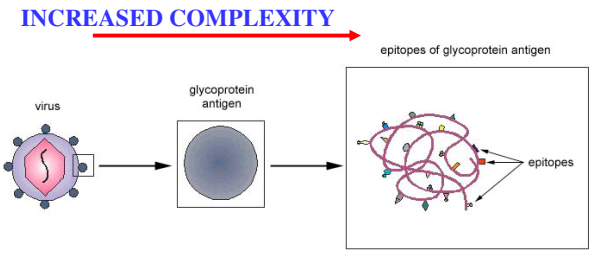
Complete Freund's Adjuvant - mineral oil-based adjuvant containing dead bacteria - increases persistence of the antigen, stimulates a chronic inflammatory response (granuloma), and co-stimulatory signals

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 antigen receptor, it is the part that determines the antigenicity of the antigen - thus the term "antigenic determinant".

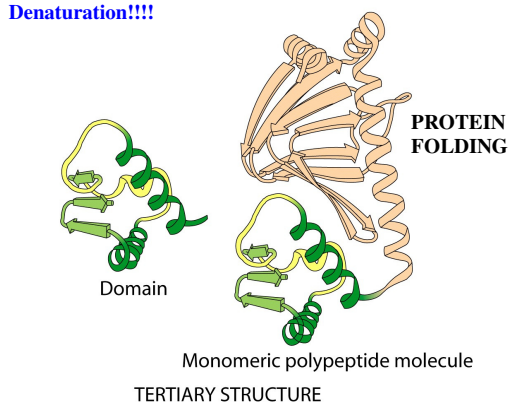
-T and B cells recognize different epitopes on an antigen



- **Each different protein and glycoprotein of a virus (or bacterium or foreign cell) constitutes a different antigen**
- **Each different antigen contains a number of different epitopes**

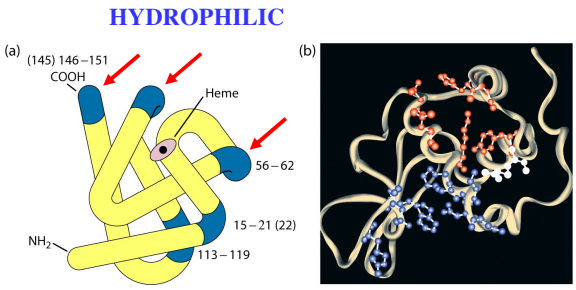
Properties of B cell epitopes (Table 3-4)

- **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.



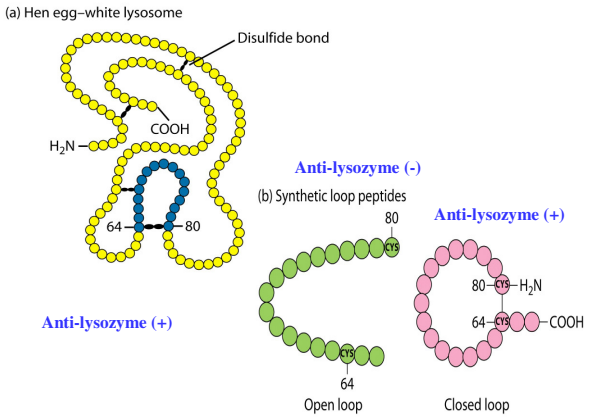
Properties of B cell epitopes (Table 3-4)

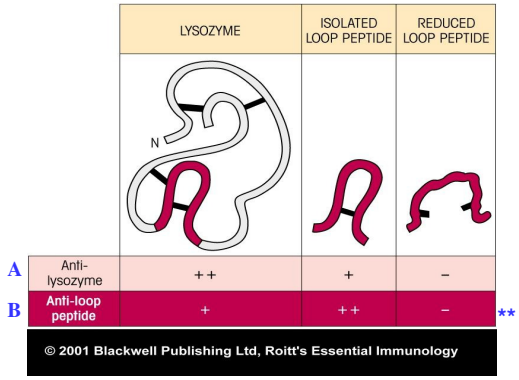
- Usually dependent on the native, tertiary conformation of the antigen
- **Must be accessible - tend to be on the "surface" of the antigen (hydrophilic)**
- May be made of sequential or non-sequential amino acid sequences (epitopes made up of non-sequential amino acid sequences are called "conformational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



Properties of B cell epitopes (Table 3-4)

- Usually dependent on the native, tertiary conformation of the antigen
- 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.

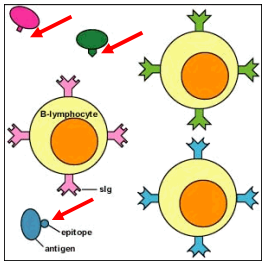


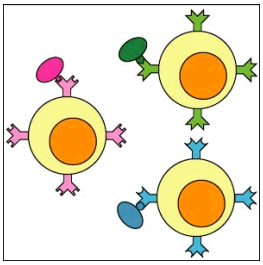


1. **Antibody binding may be lost after a protein is denatured!!**
2. **???**

Properties of B cell epitopes (Table 3-4)

- Usually dependent on the native, tertiary conformation of the antigen
- 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.





"B-lymphocytes have sIg molecules on their surface that recognize epitopes directly on antigens. Different B-lymphocytes are programmed to produce different molecules of sIg, 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)

- Usually dependent on the native, tertiary conformation of the antigen
- 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.**

Large antigens contain multiple, overlapping B cell epitopes.

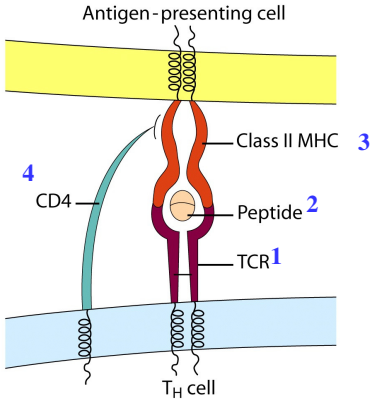
Amino acids 1-12 — Epitope 1
Amino acids 8-20 — Epitope 2
Amino acids 19-33 — Epitope 3

Linear or Sequential Antigen

Would this cause cross-reactivity?

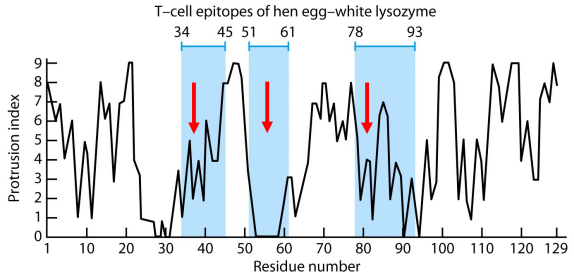
Properties of T cell epitopes (Table 3-4)

- **Involves a tertiary complex: T cell receptor, antigen, and MHC molecule**
- 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.



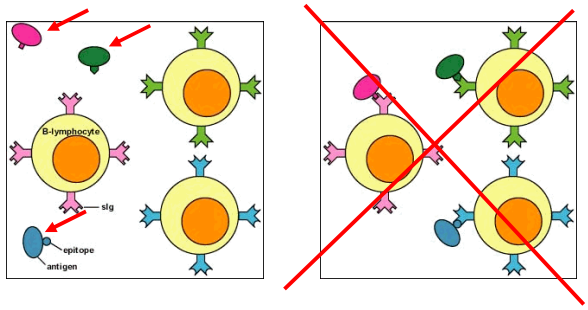
Properties of T cell epitopes (Table 3-4)

- Involves a tertiary complex: T cell receptor, antigen, and MHC molecule
- **Internal linear peptides (hydrophobic) produced by processing and bound to MHC molecules**
- Binds to soluble antigen, No MHC molecule requirement
- Large antigens contain multiple, overlapping B cell epitopes.



Properties of T cell epitopes (Table 3-4)

- Involves a tertiary complex: T cell receptor, antigen, and MHC molecule
- Internal linear peptides (hydrophobic) produced by processing and bound to MHC molecules
- **Does not bind to soluble antigen, APC processing**
- Recognize mostly proteins but some lipids and glycolipids can be presented on MHC-like molecules



Must be processed & presented with MHC in APC!!!!

Properties of T cell epitopes (Table 3-4)

- Involves a tertiary complex: T cell receptor, antigen, and MHC molecule
- Internal linear peptides (hydrophobic) produced by processing and bound to MHC molecules
- Does not bind to soluble antigen, APC processing
- **Recognize mostly proteins but some lipids and glycolipids can be presented on MHC-like molecules (remember CD1 molecules!)**

Good exam question!!!

TABLE 3-5 Antigen recognition by T and B lymphocytes reveals qualitative differences

		SECONDARY IMMUNE RESPONSE	
Primary immunization	Secondary immunization	Antibody production	Cell-mediated T_{DTH} response*
Native protein	Native protein	+	+
Native protein	Denatured protein	–	+

*T_{DTH} is a subset of $CD4^+$ T_H cells that mediate a cell-mediated response called delayed-type hypersensitivity (see Chapter 14).

Pattern-Recognition Receptors (PRR)

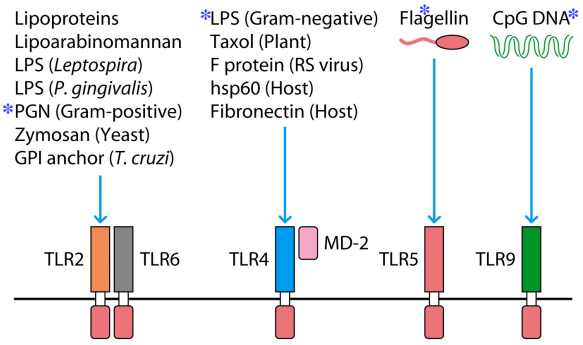
- Receptors of the innate immune system
- Recognize unique antigens (motifs) in micro-organisms (Danger Signals!!!)
- These antigens are absent in the host (non-self)
- Several Patter-Recognition Receptors (PRRs) identified
- BIO401: Toll-like receptors (TLRs)

PAMP	PRR	Biological Consequence of Interaction
Microbial cell wall components	Complement	Opsonization; Complement activation
Mannose-containing carbohydrates	Mannose-binding protein	Opsonization; Complement activation
Polyanions	Scavenger receptors	Phagocytosis
Lipoproteins of Gram + bacteria Yeast cell wall components	TLR-2 (Toll-like receptor 2)	Macrophage recruitment & activation; Secretion of inflammatory cytokines

PAMP	PRR	Biological Consequence of Interaction
Double stranded RNA	TLR-3	Production of interferon (antiviral)
LPS (lipopolysaccharide of Gram – bacteria	TLR-4	Macrophage recruitment & activation; Secretion of inflammatory cytokines

PAMP	PRR	Biological Consequence of Interaction
Flagellin (bacterial flagella)	TLR-5	Macrophage recruitment & activation; Secretion of inflammatory cytokines
CpG motifs in prokaryotes	TLR-9	Macrophage recruitment & activation; Secretion of inflammatory cytokines

Secretion of Inflammatory Cytokines activates T cells and NK cells!!!!!



Activation through TLRs → secretion of pro-inflammatory cytokines and cell recruitment