Immunogenicity Versus Antigenicity

- Substances that can be recognized by immunoglobulin receptor of B cells, or by the T-cell receptor when complexed with MHC, are called **antigens**.
- **Immunogenicity** is the ability to induce a humoral and/or cell mediated immune response
- Although a substance that induces a specific immune response is usually called an antigen, it is more appropriately called an **immunogen.**
- **Antigenicity** is the ability to combine specifically with the final products of the above responses (i.e., antibodies and/or cell-surface receptors).
- Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true.
- Some small molecules, called *haptens,* are antigenic but incapable, by themselves, of inducing a specific immune response. In other words, they lack immunogenicity.

B cells + antigen → effector B cells + memory B cells
↓
(plasma cells)

T cells + antigen → effector T cells + memory T cells
↓
(e.g., CTLs, T_Hs)

Factors That Influence Immunogenicity

- Proteins are the most potent immunogens, with polysaccharides ranking second.
- In contrast, lipids and nucleic acids of an infectious agent generally do not serve as immunogens unless they are complexed with proteins or polysaccharides.
- For cell-mediated immunity, only proteins and some lipids and glycolipids serve as immunogens.
- These molecules are not recognized directly.
- Proteins must first be processed into small peptides and then presented together with MHC molecules on the membrane of a cell before they can be recognized as immunogens.
- Immunogenicity is determined, in part, by four properties of the immunogen:
    - ✓ Foreignness
    - ✓ Molecular size
    - ✓ Chemical composition and complexity
    - ✓ Ability to be processed and presented with an MHC molecule on the surface of an antigen- presenting cell or altered self-cell

Foreignness

- In order to elicit an immune response, a molecule must be recognized as nonself by the biological system.
- When an antigen is introduced into an organism, the degree of its immunogenicity depends on the degree of its foreignness.
- Generally, the greater the phylogenetic distance between two species, the greater the structural (and therefore the antigenic) disparity between them.
- For example, the common experimental antigen bovine serum albumin (BSA) is not immunogenic when injected into a cow but is strongly immunogenic when injected into a rabbit.
- Moreover, BSA would be expected to exhibit greater immunogenicity in a chicken than in a goat, which is more closely related to bovines

Molecular size

- There is a correlation between the size of a macromolecule and its immunogenicity.
- The most active immunogens tend to have a molecular mass of 100,000 Daltons (Da).
- Generally, substances with a molecular mass less than 5000–10,000 Da are poor immunogens.
- Although a few substances with a molecular mass less than 1000 Da have proven to be immunogenic.

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

Chemical composition and heterogeneity

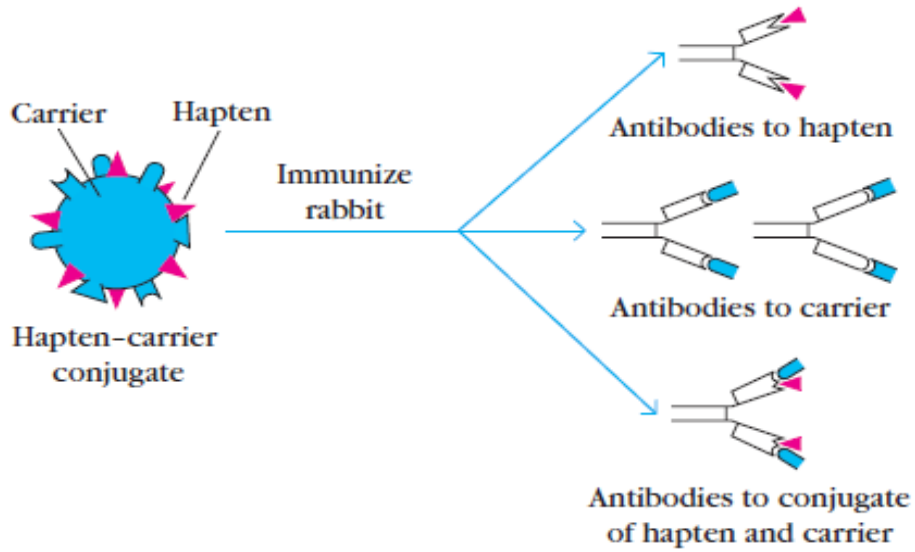
- Size and foreignness are not, by themselves, sufficient to make a molecule immunogenic; other properties are needed as well.
- For example, synthetic homopolymers (polymers composed of a single amino acid or sugar) tend to lack immunogenicity regardless of their size.
- Studies have shown that copolymers composed of different amino acids or sugars are usually more immunogenic than homopolymers of their constituents.
- These studies show that chemical complexity contributes to immunogenicity.
- In this regard it is notable that all four levels of protein organization—primary, secondary, tertiary, and quaternary—contribute to the structural complexity of a protein and hence affect its immunogenicity

Susceptibility to antigen processing and presentation

- The development of both humoral and cell-mediated immune responses requires interaction of T cells with antigen that has been processed and presented together with MHC molecules.
- Large, insoluble macromolecules generally are more immunogenic than small, soluble ones because the larger molecules are more readily phagocytosed and processed.
- Macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens.
- This can be illustrated with polymers of D-amino acids, which are stereoisomers of the naturally occurring L-amino acids.
- Because the degradative enzymes within antigen-presenting cells can degrade only proteins containing L-amino acids, polymers of D-amino acids cannot be processed and thus are poor immunogens.

Haptens

- **Haptens are** small organic molecules that are antigenic but not immunogenic.
- Chemical coupling of a hapten to a large protein, called a **carrier,** yields an immunogenic **hapten-carrier conjugate.**
- Animals immunized with such a conjugate produce antibodies specific for
    - the hapten determinant
    - unaltered epitopes on the carrier protein, and
    - new epitopes formed by combined parts of both the hapten and carrier
- By itself, a hapten cannot function as an immunogenic epitope.
- But when multiple molecules of a single hapten are coupled to a carrier protein, the hapten becomes accessible to the immune system and can function as an immunogen.



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)

FIGURE 3-10 A hapten-carrier conjugate contains multiple copies of the hapten—a small nonimmunogenic organic compound such as dinitrophenol (DNP)—chemically linked to a large protein carrier such as bovine serum albumin (BSA). Immunization with DNP alone elicits no anti-DNP antibodies, but immunization with DNP-BSA elicits three types of antibodies. Of these, anti-DNP antibody is predominant, indicating that in this case the hapten is the immunodominant epitope in a hapten-carrier conjugate, as it often is in such conjugates.

Clinical significance of Haptens

- **Many biologically important substances, including drugs, peptide hormones, and steroid hormones, can function as haptens.**
- **Conjugates of these haptens with large protein carriers can be used to produce hapten-specific antibodies.**
- **These antibodies are useful for measuring the presence of various substances in the body.**
- **For instance, the original home pregnancy test kit employed antihapten antibodies to determine whether a woman's urine contained human chorionic gonadotropin (HCG), which is a sign of pregnancy.**

- Lipids are used as haptens and attached to suitable carrier molecules such as the proteins keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA).

- By immunizing with these lipid-protein conjugates it is possible to obtain antibodies that are highly specific for the target lipids.

- Using this approach, antibodies have been raised against a wide variety of lipid molecules including steroids, complex fatty-acid derivatives, and fat-soluble vitamins such as vitamin E.

- Such antibodies are of considerable practical importance since many clinical assays for the presence and amounts of medically important lipids are antibody-based (Leukotriene C4, D4, E4, PGD2).

- Antibodies have successfully been raised against endogenous & unreactive small molecules such as some neurotransmitters (e.g. serotonin (5HT), glutamate, dopamine, GABA, tryptamine, glycine, noradrenaline), amino acids (e.g. tryptophan, 5-hydroxytryptophan, 5-methoxytryptophan), by using glutaraldehyde to crosslink these molecules to carrier proteins suitable for immune recognition.

- A well-known example of a hapten is urushiol, which is the toxin found in poison ivy. When absorbed through the skin from a poison ivy plant, urushiol undergoes oxidation in the skin cells to generate the actual hapten, a reactive quinone-type molecule, which then reacts with skin proteins to form hapten adducts. After a second exposure, the proliferated T-cells become activated, generating an immune reaction that produces typical blisters of a urushiol-induced contact dermatitis.

- Other example of a hapten-mediated contact dermatitis is nickel allergy, which is caused by nickel metal ions penetrating the skin and binding to skin proteins.

Epitopes

- Immune cells do not interact with, or recognize, an entire immunogen molecule; instead, lymphocytes recognize discrete sites on the macromolecule called **epitopes,** or **antigenic determinants.**
- Epitopes are the immunologically active regions of an immunogen that bind to antigen-specific membrane receptors on lymphocytes or to secreted antibodies.
- Studies with small antigens have revealed that B and T cells recognize different epitopes on the same antigenic molecule.
- For example, when mice were immunized with glucagon, a small human hormone of 29 amino acids, antibody was elicited to epitopes in the amino terminal portion, whereas the T cells responded only to epitopes in the carboxyl-terminal portion.
- Lymphocytes may interact with a complex antigen on several levels of antigen structure. An epitope on a protein antigen may involve elements of the primary, secondary, tertiary, and even quaternary structure of the protein.
- In polysaccharides, branched chains are commonly present, and multiple branches may contribute to the conformation of epitopes.

- The recognition of antigens by T cells and B cells is fundamentally different.
- B cells recognize soluble antigen when it binds to their membrane-bound antibody.
- Because B cells bind antigen that is free in solution, the epitopes they recognize tend to be highly accessible sites on the exposed surface of the immunogen.
- As noted previously, most T cells recognize only peptides combined with MHC molecules on the surface of antigen-presenting cells and altered self-cells; T-cell epitopes, as a rule, cannot be considered apart from their associated MHC molecules.

TABLE 3-4 Comparison of antigen recognition by T cells and B cells

Characteristic	B cells	T cells
Interaction with antigen	Involves binary complex of membrane Ig and Ag	Involves ternary complex of T-cell receptor, Ag, and MHC molecule
Binding of soluble antigen	Yes	No
Involvement of MHC molecules	None required	Required to display processed antigen
Chemical nature of antigens	Protein, polysaccharide, lipid	Mostly proteins, but some lipids and glycolipids presented on MHC-like molecules
Epitope properties	Accessible, hydrophilic, mobile peptides containing sequential or nonsequential amino acids	Internal linear peptides produced by processing of antigen and bound to MHC molecules

Properties of B-Cell Epitopes Are Determined by the Nature of the Antigen-Binding Site

- *The ability to function as a B-cell epitope is determined by the nature of the antigen-binding site on the antibody molecules displayed by B cells.*
- Antibody binds to an epitope by weak noncovalent interactions, which operate only over short distances.
- For a strong bond, the antibody's binding site and the epitope must have complementary shapes that place the interacting groups near each other. This requirement poses some restriction on the properties of the epitope.
- The size of the epitope recognized by a B cell can be no larger than the size of the antibody's binding site.
- For any given antigen-antibody reaction, the shape of the epitope that can be recognized by the antibody is determined by the shape assumed by the sequences of amino acids in the binding site and the chemical environment that they produce.
- Smaller ligands such as carbohydrates, small oligonucleotides, peptides, and haptens often bind within a deep pocket of an antibody.
- For large globular protein antigens such as hen egg-white lysozyme (HEL) and neuraminidase antibodies make contact with the antigen across a large flat face.
- The interacting face between antibody and epitope is a flat or undulating surface in which protrusions on the epitope or antibody are matched by corresponding depressions on the antibody or epitope.

- *The B-cell epitopes on native proteins generally are composed of hydrophilic amino acids on the protein surface that are topographically accessible to membrane-bound or free antibody.*
- A B-cell epitope must be accessible in order to be able to bind to an antibody; in general, protruding regions on the surface of the protein are the most likely to be recognized as epitopes, and these regions are usually composed of predominantly hydrophilic amino acids.
- Amino acid sequences that are hidden within the interior of a protein often consist of predominantly hydrophobic amino acids.
- *B-cell epitopes can contain sequential or nonsequential amino acids.*
- Epitopes may be composed of sequential contiguous residues along the polypeptide chain or nonsequential residues from segments of the chain brought together by the folded conformation of an antigen.
- Most antibodies elicited by globular proteins bind to the protein only when it is in its native conformation. Because denaturation of such antigens usually changes the structure of their epitopes, antibodies to the native protein do not bind to the denatured protein.
- *B-cell epitopes tend to be located in flexible regions of an immunogen and display site mobility*
- The major antigenic determinants in these proteins generally are located in the most mobile regions.
- The mobility of epitopes maximizes complementarity with the antibody's binding site, permitting an antibody to bind with an epitope that it might bind ineffectively if it were rigid.
- *Complex proteins contain multiple overlapping B-cell epitopes, some of which are immunodominant.*
- Within an animal, certain epitopes of an antigen are recognized as immunogenic, but others are not.
- Furthermore, some epitopes, called **immunodominant,** induce a more pronounced immune response than other epitopes in a particular animal.
- It is highly likely that the intrinsic topographical properties of the epitope as well as the animal's regulatory mechanisms influence the immunodominance of epitopes.

Antigen-Derived Peptides Are the Key Elements of T-Cell Epitopes

- In case of B cell epitope, primary immunization was with a native protein, only native protein, not denatured protein, could elicit a secondary antibody (humoral) response.
- In contrast, both native and denatured protein could elicit a secondary cell-mediated response.
- The finding that a secondary response mediated by T cells was induced by denatured protein, even when the primary immunization had been with native protein, initially puzzled immunologists.
- In the 1980s, however, it became clear that T cells do not recognize soluble native antigen but rather recognize antigen that has been processed into **antigenic peptides,** which are presented in combination with MHC molecules.
- For this reason, destruction of the conformation of a protein by denaturation does not affect its T-cell epitopes.

- *Antigenic peptides recognized by T cells form trimolecular complexes with a T-cell receptor and an MHC molecule.*
- Unlike B-cell epitopes, which can be viewed strictly in terms of their ability to interact with antibody, T-cell epitopes must be viewed in terms of their ability to interact with both a T-cell receptor and an MHC molecule.
- *Antigen processing is required to generate peptides that interact specifically with MHC molecules.*
- *E*ndogenous and exogenous antigens are usually processed by different intracellular pathways.
- Endogenous antigens are processed into peptides within the cytoplasm, while exogenous antigens are processed by the endocytic pathway.
- *Epitopes recognized by T cells are often internal*.
- T cells tend to recognize internal peptides that are exposed by processing within antigen-presenting cells or altered self-cells.
- The tertiary conformation of hen egg-white lysozyme and sperm whale myoglobin to determine which amino acids protruded from the natural molecule.
- Mapping of the major T-cell epitopes for both proteins revealed that, in each case, the T-cell epitopes tended to be on the “inside” of the protein molecule