ANTIGEN

1. Antigenicity and immunogenicity, Immunogens, Adjuvants and haptens
2. Factors influencing immunogenicity
3. B and T-Cell epitopes

Substacens that can be recognized by the immunoglobulin receptor of B cells, or by the T- cell receptor when complexed with MHC, are called **antigen.**

ANTIGENICITY AND IMMUNOGENICITY

Immunogenicity and antigenicity are related but distinct immunologic properties that sometimes are confused. Immunogenicity is the ability to induce a humoral and/or cell mediated immune response:

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)

Antigenicity is the ability to combine specifically with 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.

To protect against infectious disease, the immune system must be able to recognize bacteria, bacterial products, fungi, parasites, and viruses as immunogens. In fact, the immune system actually recognizes particular macromolecules of an infectious agent, generally either proteins or polysaccharides. **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. 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.

FACTORS INFLUENCING IMMUNOGENICITY

Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that the antigen encounters. Immunogenicity is determined, in part, by four properties of the immunogen:

FOREIGNNESS

In order to elicit an immune response, a molecule must be recognized as non-self by the biological system. The capacity to recognize nonself is accompanied by tolerance of self. Tolerence is specific unresponsiveness to self antigens. Ability to tolerate self antigens arises during lymphocyte development, during which immature lymphocytes are exposed to self-components. Antigens that have not been exposed to immature lymphocytes during this critical

period may be later recognized as nonself, or foreign, by the immune system. 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. Some self-components (e.g., corneal tissue and sperm) if injected even into the animal from which they originated, they will function as immunogens.

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 kD. Generally, substances with a molecular mass less than 5–10 kD are poor immunogens, although a few substances with a molecular mass less than 1 kD have proven to be immunogenic.

CHEMICAL COMPOSITION AND HETEROGENEITY

Chemical complexity contributes to immunogenicity. 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. 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. 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.

ADJUVANTS

Adjuvants (from Latin adjuvare, to help) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen. Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen are available. For example, the antibody response of mice to immunization with BSA can be increased fivefold or more if the BSA is administered with an adjuvant. Adjuvants appear to exert one or more of the following effects

■ Antigen persistence is prolonged. ■ Co-stimulatory signals are enhanced. ■ Local inflammation is increased. ■ The nonspecific proliferation of lymphocytes is stimulated.

Water-in-oil adjuvants prolong the persistence of antigen. A preparation known as **Freund's incomplete adjuvant** contains antigen in aqueous solution, mineral oil, and an emulsifying agent such as mannide monooleate, which disperses the oil into small droplets surrounding the antigen; the antigen is then released very slowly from the site of injection. **Freund's complete adjuvant** contains heat-killed Mycobacteria as an additional ingredient. Muramyl dipeptide, a component of the mycobacterial cell wall, activates macrophages, making Freund's complete adjuvant far more potent than the incomplete form.

EPITOPE: T CELL AND B CELL EPITOPES

Epitopes are the immunologically active regions of an immunogen that bind to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. 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.**

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.

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. 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

- 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. The size of the epitope recognized by a B cell can be no larger than the size of the antibody's binding site. Smaller ligands such as carbohydrates, small oligonucleotides, peptides, and haptens often bind within a deep pocket of an antibody. For example, angiotensin II, a small octapeptide hormone, binds within a deep and narrow groove (725 Å2) of a monoclonal antibody specific for the hormone. All eight amino acid residues of the octapeptide are in van der Waals contact with 14 residues of the antibody's groove.

- 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**. Amino acid sequences that are hidden within the interior of a protein often consist of predominantly hydrophobic amino acids, and cannot function as B-cell epitopes unless the protein is first denatured. In the crystallized antigen-antibody complexes it has been shown that between 15 and 22 amino acids on the antigen contact the antibody by 75–120 hydrogen bonds as well as by ionic and hydrophobic interactions.
- B-cell epitopes can contain **sequential or nonsequential amino acids.**

TYPES OF EPITOPE OR DETERMINANTS FOR ANTIBODY

Linear determinants:

- **Epitopes** formed by several adjacent amino acid residues.
- The antigen binding site of an antibody can usually accommodate a linear determinant made up of about 6 amino acids.
- If linear determinants appear on external surface or in a region of extended conformation in the native folded protein, they may be accessible to antibodies. Linear determinants may be inaccessible in the native conformation and appear only when the protein is denatured.

Conformational determinants: Formed by amino acid residues that are not in a sequence but become spatially juxtaposed in the folded protein.

Antibodies specific for certain linear determinants and antibody specific for conformational determinants can be used to ascertain whether a protein is denatured or in its native conformation, respectively.

Neoantigenic determinants: Proteins may be subjected to modifications such as phosphorylation or proteolysis. These modifications, by altering the covalent structure, can produce new epitopes and they too may recognized by specific antibodies.

- **B-cell epitopes tend to be located in flexible regions of an immunogen and display site mobility**. The major antigenic determinants in proteins generally were located in the most mobile regions. Site 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.

AFFINITY AND AVIDITY

The strength of the binding between a single combining site of an antibody and an epitope of an antigen is called **affinity** of the antibody. The affinity is commonly represented by a **dissociation constant (Kd)**, which indicates the concentration of antigen that is required to occupy the combining sites of half the antibody molecules present in a solution of antibody. A small Kd indicates a stronger or higher affinity interaction because a lower concentration of

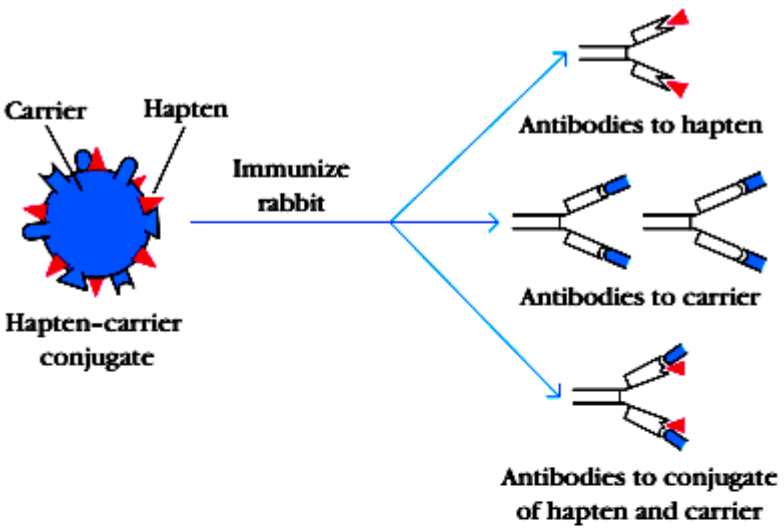
antigen is needed to occupy the sites. The Kd of antibodies produced in typical humoral responses usually varies from about 10^{-7} - 10^{-11} M.

The affinity of any one antigen-binding site will be the same for each epitope of a polyvalent antigen, the strength of attachment of the antibody to the antigen must take into account binding of all the sites of antibodies present to all the available epitopes. This overall strength of attachment is called **avidity**. Avidity is much greater than the affinity of any one antigen binding site. Mathematically the avidity increases almost geometrically (rather than additively) for each occupied site. Thus a low affinity IgM molecule can still bind tightly to a polyvalent antigen because many low affinity interaction upto 10 per IgM molecule) can produce a single high avidity interaction.

HAPTEN

Haptens are small organic molecules that are antigenic but not immunogenic. Karl Landsteiner in the 1920s and 1930s created a simple, chemically defined system for studying the binding of an individual antibody to a unique epitope on a complex protein antigen. Chemical coupling of a hapten to a large carrier protein yields an immunogenic **hapten-carrier conjugate**. Animals immunized with such a conjugate produce antibodies specific for (1) the hapten determinant, (2) unaltered epitopes on the carrier protein, and (3) 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.

Significance: 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.



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.

PROPERTIES OF T CELL EPITOPES

Immunization with a native protein not denatured protein, could elicit a antibody (humoral) response. In contrast, both native and denatured protein could elicit a

secondary cell-mediated response. 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.
- The binding of an MHC molecule to an antigenic peptide does not have the fine specificity of the interaction between an antibody and its epitope. Instead, a given MHC molecule can selectively bind a variety of different peptides. For example, the class II MHC molecule can bind peptides from ovalbumin (residues 323–339), hemagglutinin (residues 130–142), and lambda repressor (residues 12–26). Structural features, or motifs, common to different peptides that bind to a single MHC molecule.
- Antigen processing is required to generate peptides that interact specifically with MHC molecules. 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.