# Antigens and Immunogens

## Introduction

I**mmunogen** is any agent capable of inducing an immune response.

**Antigen** is any agent capable of binding specifically to components of immune response such as lymphocytes and antibodies.

The distinguishing between terms is necessary because there are many compounds that are capable of binding with components of immune system that have been induced specifically against them.

**Thus all immunogens are antigens, but not all antigens need to be immunogens**. This difference become obvious in the case of low molecular weight compounds, a group of substances includes many antibiotics and drugs.

**Haptens** are low molecular weight compounds that can combine with antibody but cannot initiate an immune response unless it is coupled to a larger **carrier** molecule.

For example, **nickel** is a substance of small molecular weight which is incapable of provoking an immune response in its own right. **Nickel allergy**, however, is a common cause of contact dermatitis. This results when nickel combines with protein in the patient's skin. The nickel-protein complex is recognized as foreign and an immune response is mounted.

## Requirements for immunogenicity

A substance must possess the following three characteristics to be immunogen. 1- Foreignness

2- High molecular weight

3- Chemical complexity.

| Parameter | Increase immunogenicity | Decreased immunogenicity |
|---|---|---|
| Size | Large | Small (MW<2500) |
| Composition | Complex | Simple |
| Similarity to self protein | Multiple differences | Few differences |
| Interaction with host MHC | Effective | Ineffective |

**Table 3: Intrinsic properties of proteins that affect immunogenicity.**

### Foreignness

The immune system of an individual can normally distinguish between body components, i.e. **'self'** and foreign substances, **'non-self'**. Normally, the body is tolerant to its own components, and does not initiate an immune response against these.

### High molecular weight

Small molecules such as amino acids or monosaccharides are usually not antigenic. As a rule, molecules with a molecular weight of less than 10,000 have no or only weal antigenicity. However, as mentioned above, if coupled to a suitable carrier molecule such as a protein, low molecular weight substance (haptens) can exhibit antigenicity.

## Chemical complexity

The configuration and complexity of the molecule are important. Linear polypeptides and globular proteins are both capable of inducing an immune response.

Antibody that is formed to these different structures is highly specific and when the conformation of an antigen is changed the antibody induced by the original form no longer combines with it.

For example, it is possible for an individual to produce and immune response to raw egg antigens, but when the egg is boiled the antigenic configuration is changed and no immune response is mounted. The need for complexity means that molecules containing a repeating unit of only one amino acid are generally poor antigens, even if the molecule is large.

## Further requirements for antigenicity

In addition to the above characteristics several other factors play roles in the determining whether a substance is immunogenic. These include the susceptibility of the substance to **enzymatic degradation** and the **genetic make up** of the host, Table 4.

| Parameter | Increased immunogenicity | Decreased immunogenicity |
|---|---|---|
| Dose | Intermediate | High or low |
| Route | Subcutaneous > intraperitoneal > intravenous or intragastric | |
| Form | Particulate | Soluble |
| | Denatured | Native |
| Adjuvants | Slow release | Rapid release |
| | Bacteria | No bacteria |

**Table 4: Factors that influence the adapted immunoresponse to an antigen.**

To activate T cell, the substance must be susceptible to partial enzymatic degradation that takes place during antigen processing and presentation by antigen presenting cells such as macrophage.

**Genetic factors** also play a part. Not all individuals within a species will show the same response to a substance - some are responders and some are non responders. Likewise, there is a wide variation between species.

The **method of administration** and the **dose** are also important. The immune response to a substance can be enhanced by administering an **adjuvant*** together with the antigen, while a state of immunological unresponsiveness can result if a very high or low doses of certain antigens are administered.

## Antigenic determinants

Despite the fact that potent antigens are relatively large molecules, only limited parts of the molecule are involved in the binding to antibodies. These parts are called **antigenic determinants or epitopes**. A molecule must have at least two antigenic determinants in order to stimulate antibody production.

### Epitopes recognized by B cells and T cells

**B cells** with their membrane-bound antibody, which serve as epitope receptors, recognize and bind **free antigen in solution.** Thus, the epitopes on the antigen must be on the 'outside' of the molecule, accessible for interaction with the receptor.

* An adjuvant is a substance injected with antigens which non-specifically enhances the immune response to that antigen.

On the other hand, the interaction of epitope with the T-cell receptor requires prior 'processing' of the antigen, and the association of an area of the processed antigen with MHC molecules present on the surface of the antigen-presenting cell. Generally such 'processed' epitopes are internal denatured proteins. Polysaccharides contain solely B-cell recognizable epitopes, Figure 9. With respect to their epitopes, antigens may have the characteristics shown schematically in Figure 9.

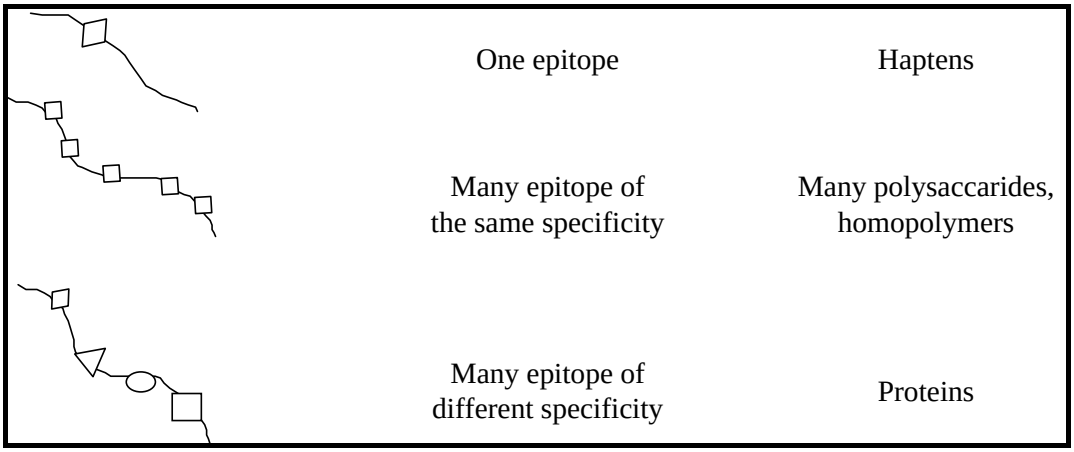


**Figure 9: Representation of some possible antigenic structures.**

Thus, they may consist of a single epitope (hapten) or have varying numbers of the same epitope on the same molecule (polysaccharides). The most common antigens (proteins) have varying numbers of different epitopes on the same molecule.

## Major classes of antigens

The following major chemical families may be antigenic:

**Carbohydrates (polysaccharides):** polysaccharides are potentially, but not always, immunogenic. Antibodies, can be induced against many kinds of polysaccharide molecules, such as components of microorganisms. An excellent example of antigenicity of polysaccharides is the immune response associated with the ABO blood groups, which are polysaccharide on the surface of the red blood cells.

**Lipids**: lipids are rarely immunogenic, but an immune response to lipids may be induced if the lipids are conjugated to protein carriers.

**Nucleic acids:** nucleic acids are poor immunogens by themselves, but they become immunogenic when they are conjugated to protein carriers.

**Proteins**: virtually all proteins are immunogenic. Thus, the most common immune responses are those to proteins. Furthermore, the greater the degree of complexity of the protein, the more vigorous will be the immune response to that protein. In general, proteins are multi-determinant antigens.

## Cross reactivity

The binding of the antigenic determinant to the antibody binding site can be linked to a **'lock and key'** situation, Figure 10. The most efficient immunological responses occur when the antigen and antibody fit exactly.

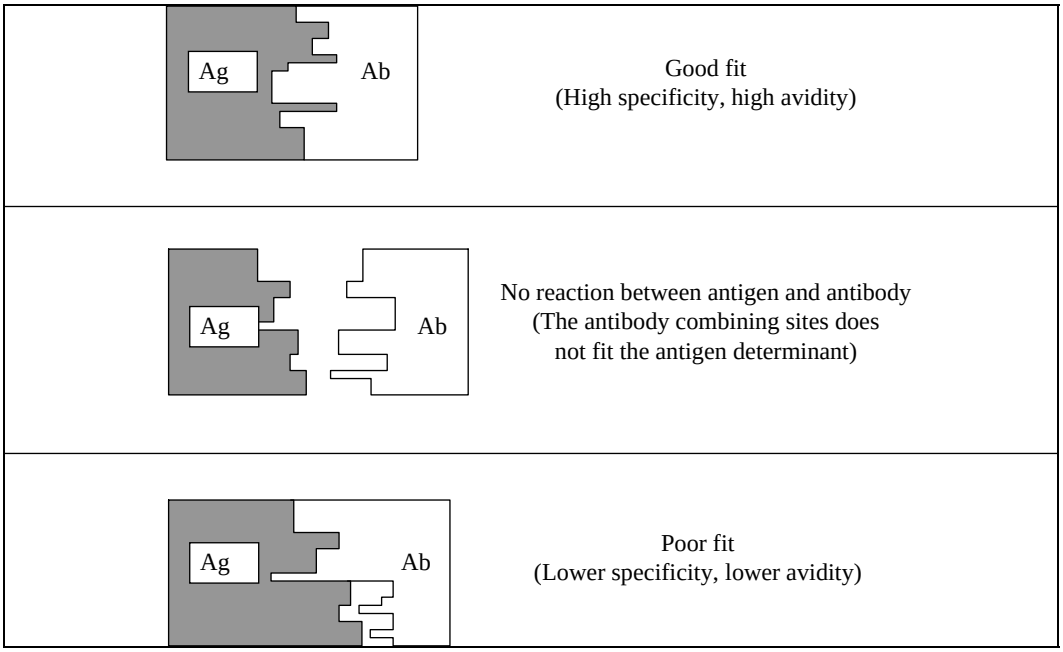


**Figure 10: Antigen-antibody binding.**

Antibodies of different degrees of specificity may be produced in the immune response to a given antigen. Sometimes an antigen can combine as a **'poor fit'** with an antibody that was produced in response to an entirely different antigen. This is demonstrated in the phenomenon of **cross-reactivity**.

In the course of some infections, antibody produced to the microorganism in question can produce a 'poor fit' with the host's own tissue antigens and immunological damage may result. For example, in acute rheumatic fever, it is thought that antibody produced against *Streptococcus pyogenes* in the throat cross-react with the host's heart tissue leading to myocarditis and valvular disease.

This concept is important in relation to immunization against highly pathogenic microorganisms or highly toxic compounds. **A toxin** that has been modified to the extent that it is no longer toxic but still maintains some of its immunochemical characteristics is called a **toxoid**.

Thus we can say that a toxoid cross-reacts immunologically with the toxin. Accordingly, it is possible to immunize individuals with the toxoid and thereby induce immune responses to some of the epitopes that the toxoid still shares with the native toxin, because these epitopes have not been destroyed by the modification.

Although the molecules of toxin and toxoid differ in many physicochemical and biological respects, they nevertheless cross-react immunologically; they share enough epitopes to allow the immune response to the toxoid to mount an effective defense against the toxin itself.

## Immunological adjuvants

To enhance the immune response to a given immunogen, various **additives** or **vehicles** are often used. An **adjuvant** is a substance that, when mixed with an immunogen, enhances the immune response against the immunogen.

| Adjuvant name | Composition | Mechanism of action |
|---|---|---|
| Incomplete Freund's adjuvants | Oil in water emulsion | Delayed release of antigen; enhanced uptake by macrophage |
| Complete Freund's adjuvants | Oil in water emulsion with dead mycobacteria | Delayed release of antigen; enhanced uptake and induction of co-stimulators in macrophages |

**Table 5: Commonly used adjuvants.**

It is important to distinguish between a carrier for a hapten and an adjuvant. A hapten will become immunogenic when conjugated covalently to a carrier; it will not become immunogenic if mixed with an adjuvant. Thus, an adjuvant enhances the immune response to immunogens but does not confer immunogenicity to haptens.

The most widely used adjuvant in humans is **alum precipitate**, a suspension of aluminum hydroxide on which the antigen is absorbed. This adjuvant causes **aggregation** of a soluble antigen and allows continuos **slow release** of antigen. In addition, it has a slight irritant effect that enhances the ingestion and processing of an antigen by macrophages which present the antigen to T cells, leading to **T-cell activation.**