INTRODUCTION TO IMMUNOLOGY

Immunoassays are diagnostic procedures generally performed in the same way as chemical tests. The major reagents are antigens and antibodies utilizing immunological reactive principles. The antibodies are formed directly in animals or are prepared as monoclonal antibodies. The latter are derived from cells of the immunological system grown in cell cultures. Thus, it is important to understand the mammalian immunological system.

The reaction between antibody and its antigen, which is the heart of the immunodiagnostic test, is best understood in terms of the immunology involved. For these reasons, the next section will discuss the immunological basis for immunodiagnostic test procedures.

Antigens

An antigen is a substance that causes an animal to produce antibodies. The antigen must be in direct contact with certain cells of the animal, which means that usually it must be introduced into the animal's blood stream.

Antigens are given subcutaneously, intravenously, intramuscularly, intraperitoneally, intradermally, or by inhalation. In exceptional cases the antigen can be introduced by mouth. This is effective only if the digestive process does not destroy the antigen before it comes into contact with the appropriate cells.

An antigen elicits the immune response, creating immunity and protecting the host animal against an invading organism. Most antigens important in immunoassays bear no threat to the animal. For our purposes, the host animal can be viewed merely as a means of producing a desired antibody. Herein, emphasis is on the use of antigens and antibodies as chemical reagents rather than on their role in the immune response.

To cause an animal to produce antibodies against an antigen, the antigen molecule must have certain properties.

- Size: It must be a minimum size, usually with a molecular weight of 5,000 or more. The higher the molecular weight, the more likely the molecule will act as an antigen.

- Complexity: The molecule must have a high level of internal chemical complexity. Large natural proteins and polysaccharides are among the most antigenic substances known. Yet even extremely large polymers of common plastics are not antigenic because they lack complexity; or, to be more precise, they lack certain types of chemical groups that are antigenic.

- Solubility: The molecule must be soluble in body fluids.

- Foreignness: The molecule must be foreign to the host animal. It

must come either from a different species or from the same species but with a different antigenic makeup. This implies that the antigen molecule has a different molecular composition, at least in the antigenic determinant site, or epitope. If we did not have a mechanism to distinguish our proteins from those from outside, we would continuously and disastrously react against our own proteins.

To elicit antibody production, the optimal amount of antigen must be given to the animal. Too little will not elicit a response. Too much will overwhelm the animal's immune system, and the animal will become inactive regarding that antigen. It will not produce antibody against that antigen.

Common antigens are listed in Table I. Nearly all large proteins are good antigens, as are most high molecular weight polysaccharides. Nucleic acids, such as DNA and RNA, have a high molecular weight but are poor

Table I

Some Antigenic Substances

Proteins	Molecular Weight	Polysaccharides
Ribonuclease	14,000	Blood group substances
Virus proteins	15,000 and up	Dextrans
Egg albumin	40,000	
Tetanus toxin	66,000	
Thyroglobulin	669,000	
Serum proteins		
Albumins		
Globulins		

antigens. Although they are complex molecules in terms of the genetic information they encode, their three-dimensional structure is too simple to elicit an immune response.

Epitopes. An epitope is the portion of an antigen molecule that elicits the antibody response. An antigen molecule usually has more than one epitope, each occupying a small part of the molecule. The three dimensional configuration of the epitope directs the cell in making an antibody molecule, which reacts specifically with its own epitope, as Figure 2 shows.

INSERT FIGURE 2

Figure 2. Antigenic determinants. (A) Antigenic determinants on two different antigens; (B) antigenic determinants on a single molecule (antigen with two epitopes); (C) hapten coupling with a carrier protein molecule, thereby becoming antigenic.

Haptens. Small molecules with a molecular weight less than 5,000 are known as haptens. They are not antigenic. In other words, they do not

elicit an antibody response when injected into an animal.

When a hapten is coupled with a large molecule, such as a protein (bovine serum albumin often is used), the complex becomes antigenic. The animal responds to the injected complex by producing antibodies against the small molecule and its bound protein. Thus, bound haptens provide large molecules with new antigenic determinant sites.

To elicit an antigenic response as part of a complex, the large molecule need not be capable of provoking the response on its own. For example, it could be a serum protein normal to the animal. The animal would not recognize the protein molecule as foreign and would not react to it.

Coupled with the hapten, however, the molecule becomes "foreign" and reactive. When haptens are injected directly into an animal, they some times couple with serum or tissue proteins. This creates a new antigen against which the animal makes antibodies. Unfortunately, some antibodies also react to the "link" between the hapten and the large molecule. These antibodies are useless for free hapten. After a hapten connected to a large molecule has been injected into an animal, the hapten alone when injected again can cause an antibody reaction.

Thyroid and steroid hormones and nearly all drugs and antibiotics have low molecular weights and are classified as haptens.

The diagnostic laboratory frequently is called upon to measure the level of a small molecule in a body fluid. Immunoassay requires the ability to make antibodies against the small molecule. Therefore, the possibility of binding a small hapten molecule to a larger carrier molecule is an important consideration. The antibody produced will bind with free hapten, which is necessary for method development. This factor must be considered in the production of diagnostic antibodies.

The_Immune_System

The immune system of higher animals has many different groups of cells scattered throughout the body. When antigens are introduced, as during an infection, these cells act as an integrated system. Chemical signals released by various cells influence other cells and keep the system working together.

Cellular_Components

The bone marrow of mammals is located in the hollow centers of long bones and plate-like bones of the skull. The marrow is responsible for hematopoiesis, which is the development and maturation of blood cells. Reticulum cells of the marrow differentiate into precursor cells, and these in turn differentiate into various blood cells, as Figure 3 illustrates.