TOPIC: Antigens Lecture #2

TEACHING OBJECTIVES:

1. To compare and contrast the immunogen, antigen, and hapten
2. To describe the factors influencing immunogenicity
3. To define the chemical nature of immunogens
4. To compare the structures of T-independent and T-dependent antigens
5. To introduce the concept of hapten-carrier conjugates and to describe their structure
6. To characterize antigenic determinants
7. To define superantigens

REQUIRED READING:

Male, *et al*. Immunology, 7th Ed., pp10-13, 165-167.

ANTIGENS

1) Definitions

a) Immunogen - a substance that induces a specific immune response.
b) Antigen (Ag) - a substance that reacts with the products of a specific immune response.
c) Hapten - a substance that is nonimmunogenic but which can react with the products of a specific immune response. Haptens are small molecules which could never induce an immune response when administered by themselves but which can when coupled to a carrier molecule. Free haptens, however, can react with products of the immune response after such products have been elicited. Haptens have the property of antigenicity but not immunogenicity.
d) Epitope or Antigenic Determinant - the portion of an antigen that combines with the products of a specific immune response.
e) Antibody (Ab) - a specific protein which is produced in response to an immunogen and which reacts with an antigen.

2) Factors influencing immunogenicity

a) Contribution of the immunogen: The immune system normally discriminates between self and non-self such that only **foreign** molecules are immunogenic. There is no absolute **size** above which a substance will be immunogenic. However, in general, the larger the molecule the more immunogenic it is likely to be. In general, the more **complex** the substance is chemically the more immunogenic it will be. The antigenic determinants are created by the primary sequence of residues in the polymer and/or by the secondary, tertiary or quaternary structure of the molecule. The **physical form**, such as whether it is particulate or soluble can affect immunogenicity. In general, particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form. Antigens that are easily **degradable** and phagocytosed are generally more immunogenic. This is because for most antigens (T-dependant antigens, see below) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

b) Contribution of the Biological System: **Genetic** factors of the host can determine immunogenicity of an antigen. Some substances are immunogenic in one species but not in another. Similarly, some substances are immunogenic in one individual but not in others (*i.e.* responders and non-responders). The species or individuals may lack or have altered genes that code for the receptors for antigen on B cells and T cells or they may not have the appropriate genes needed for the APC to present antigen to the helper T cells. **Age** can also influence immunogenicity. Usually the very young and the very old have a diminished ability to mount an immune response in response to an immunogen.

c) Method of Administration: The **dose** of administration of an immunogen can influence its immunogenicity. There is a dose of antigen above or below which the immune response will not be optimal. For example, high doses of antigen can often be tolerogenic and blunt the immune response. Generally the subcutaneous **route** is for effective for inducing an immune response than the intravenous or intragastric routes. The route of antigen administration can also alter the nature of the response. Substances that can enhance the immune response to an immunogen are called **adjuvants**. The use of adjuvants, however, is often hampered by undesirable side effects such as fever and inflammation.

3) Chemical nature of immunogens

a) The vast majority of immunogens are **proteins**. These may be pure proteins or they may be glycoproteins or lipoproteins. In general, proteins are usually very good immunogens. Pure **polysaccharides** and lipopolysaccharides are good immunogens. **Nucleic acids** are usually poorly immunogenic. However they may become immunogenic when single stranded or when complexed with proteins. In general **lipids** are non-immunogenic, although they may be haptens. Some glycolipids and phospholipids can stimulate T cells and produce a cell-mediated immune response.

4) Types of antigens

a) **T-independent** antigens are antigens which can directly stimulate the B cells to produce antibody without the requirement for T cell help. In general, polysaccharides are T-independent antigens. The responses to these antigens differ from the responses to other antigens. These antigens are characterized by the same antigenic determinant repeated many times as illustrated in Figure 1a. Many of these antigens can activate B cell clones specific for other antigens (**polyclonal activation**). T-independent antigens can be subdivided into Type 1 and Type 2 based on their ability to polyclonally activate B cells. Type 1 T-independent antigens are polyclonal activators while Type 2 are not. T-independent antigens are generally more resistant to degradation and thus they persist for longer periods of time and continue to stimulate the immune system.

b) **T-dependent** antigens are those that do not directly stimulate the production of antibody without the help of T cells. Proteins are T-dependent antigens. Structurally these antigens are characterized by a few copies of many different antigenic determinants as illustrated in the Figure 1b.

c) **Hapten-carrier conjugates** are immunogenic molecules to which haptens have been covalently attached. The immunogenic molecule is called the carrier. Structurally these conjugates are characterized by having native antigenic determinants of the carrier as well as new determinants created by the hapten (haptenic determinants) as illustrated in the Figure 1c. The actual determinant created by the hapten consists of the hapten and a few of the adjacent residues, although the antibody produced to the determinant will also react with free hapten. In such conjugates the type of carrier determines whether the response will be T-independent or T-dependent.

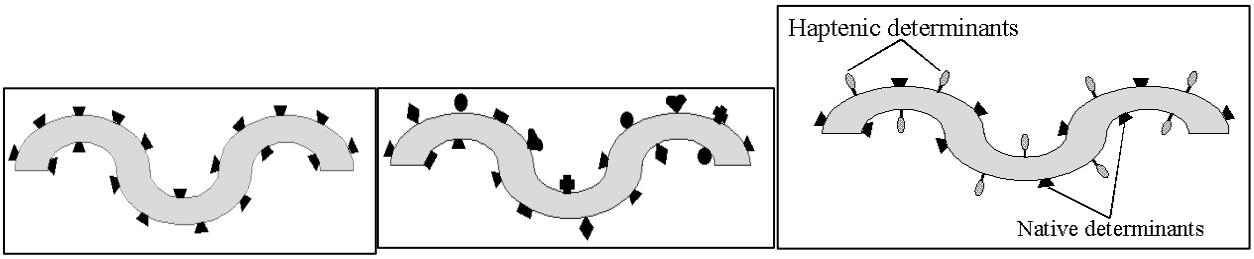

Fig 1A. **B.** **C.**

5) Antigenic determinants recognized by B cells and Ab

a) Antigenic determinants recognized by B cells and the antibodies secreted by B cells are created by the primary sequence of residues in the polymer (**linear or sequence determinants**) and/or by the secondary, tertiary or quaternary structure of the molecule (**conformational determinants**). In general antigenic determinants are small and are limited to approximately 4-8 residues. (amino acids and or sugars). The combining site of an antibody will accommodate an antigenic determinant of approximately 4-8 residues. Although, in theory, each 4-8 residues can constitute a separate antigenic determinant, in

practice, the number of antigenic determinants per antigen is much lower than what would theoretically be possible. Usually the antigenic determinants are limited to those portions of the antigen that are accessible to antibodies as illustrated in the Figure 2 (antigenic determinants are indicated in black).

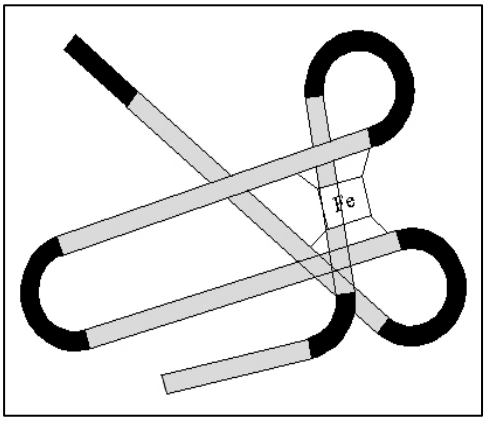

Figure 2

6) Determinants recognized by T cells

a) Antigenic determinants recognized by T cells are created by the primary sequence of amino acids in proteins. T cells do not recognize polysaccharide or nucleic acid antigens. This is why polysaccharides are generally T-independent antigens and proteins are generally T-dependent antigens. The determinants need not be located on the exposed surface of the antigen since recognition of the determinant by T cells requires that the antigen be proteolytically degraded into smaller peptides. Free peptides are not recognized by T cells, rather the peptides associate with molecules coded for by the major histocompatibility complex (MHC) and it is the complex of MHC molecules + peptide that is recognized by T cells. Some T cells can recognize lipids in conjunction with a MHC-like molecule called CD1. In general antigenic determinants are small and are limited to approximately 8-15 amino acids. Although, in theory, each 8-15 residues can constitute a separate antigenic determinant, in practice, the number of antigenic determinants per antigen is much less than what would theoretically be possible. The antigenic determinants are limited to those portions of the antigen that can bind to MHC molecules. This is why there can be differences in the responses of different individuals.

7) Superantigens

a) When the immune system encounters a conventional T-dependent antigen, only a small fraction (1 in 10^4 -10^5) of the T cell population is able to recognize the antigen and become activated (monoclonal/oligoclonal response). However, there are some antigens which polyclonally activate a large fraction of the T cells (up to 25%). These antigens are called **superantigens** (Figure 3). Examples of superantigens include: Staphylococcal enterotoxins (food poisoning), Staphylococcal toxic shock toxin (toxic shock syndrome), Staphylococcal exfoliating toxins (scalded skin syndrome) and Streptococcal pyrogenic

exotoxins (shock). Although the bacterial superantigens are the best studied there are superantigens associated with viruses and other microorganisms as well. The diseases associated with exposure to superantigens are, in part, due to hyper activation of the immune system and subsequent release of biologically active cytokines by activated T cells.

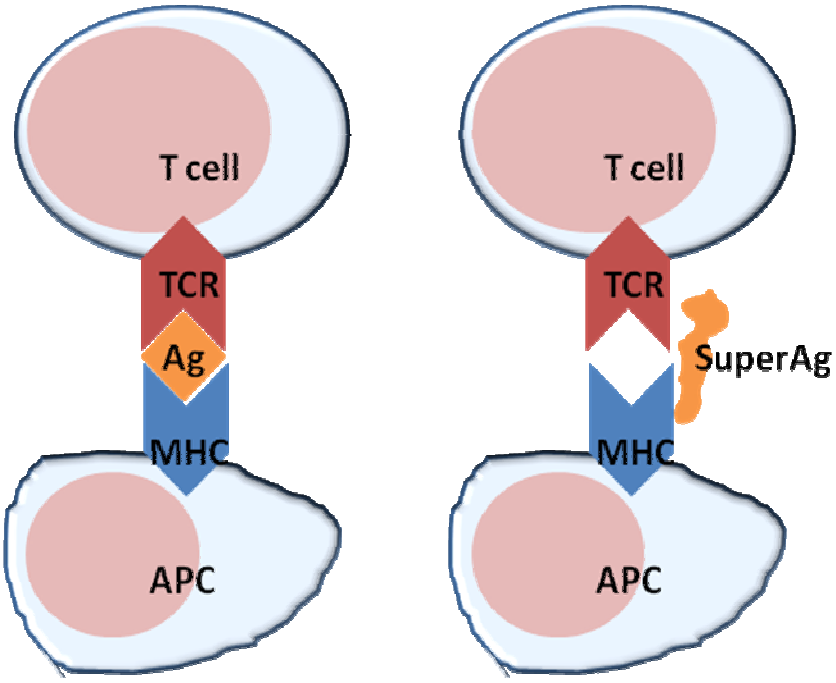

Figure 3