ANTIGENS AND ANTIGENIC DETERMINANTS

Antigens: - these are compounds which are capable of reacting with an antibody i.e. substance that reacts with the products of a specific immune response without necessarily being capable of inducing antibody formation.

Immunogen is used to describe substances that induces a specific immune response i.e. substances capable of eliciting Ab formation when injected into a host.

Generally however antigens or immunogens can be used interchangeably to refer to is a molecule which is capable of stimulating specific immunity.

Antigens could be proteins (syntheticpolypeptides, lipoproteins, and glycoproteins), polysaccharides (including lipopolysaccharides), nucleic acids or lipids. This includes parts coats, capsules, cell walls, flagella, fimbrae, and toxins) of bacteria, viruses, and other microorganisms as well as non-microbial such as pollen, egg white, morphine etc

All cells of the body possess Ags on their surface which acts as markers to help cells recognize each other however "Self" antigens are usually tolerated by the immune system; whereas "Non-self" antigens are identified as intruders and attacked by the immune system.

Immunogenicity is the ability to induce a humoral (antibody production) and/or cell-mediated immune response.
Antigenicity is the ability to combine specifically with the final products of the immune response.

Hapten:-is a chemically defined determinant that when conjugated to an immunogenic carrier stimulates synthesis of antibodies specific for that hapten. 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.

Epitope:-is the unique region on an Ag that will bind a complementary Ab i.e. that portion of an antigen that combines with the products of a specific immune response. It is also called antigenic determinant. Epitopes generally are significantly smaller than the antigens which contain them (and much smaller than the size of an antibody).A single antigen often contains numerous Epitopes. Antigenic determinants react with Abs in a lock and key fashion based on structural complementarity. Forces characteristic of Ag-Ab binding include Van Der Waal-London dipole interactions, hydrophobic interaction and ionic Columbic bonding, these cooperate together between the Epitopes of the Ags and the variable Fab regions of the Abs to form immune complex. The other [portion of the Ag other than the Epitope are termed immunogenic carrier.

Antigens enter the body through any of the following routes- ingestion, inhalation or injection.

PROPERTIES OF ANTIGENS OR IMMUNOGENS

1. Foreignness- the body can distinguish its own antigen from foreign ones, (having gotten used to overstimulation by the body's antigen) it does not produce antigens against itself but does readily to a new Ag that is introduced into the body. It has been observed that excess stimulation of the immune system by an Ag can lead to an immunologic paralysis where no Ab is mounted and this can be used to explain why the body does not mount Abs against its own Ags which are constantly present to stimulate it.
2. There are areas of structural stability within the molecule.
3. Size-a minimal molecular wt of 4000 to 5000Da, although 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.
4. The compound should have ability to be metabolized or degradability, Ags that are easily phagocytosed are generally more immunogenic.
5. Randomness of structure-
6. **Physical form-**In general particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form.
7. 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.
8. Accessibility to the immunogenic configuration of the Ab forming mechanism.
9. Affinity is a property of an Ag that refers to the energy of interaction between a single Ab combining site and corresponding Epitope on the Ag.

TYPES OF ANTIGENS

Ags can be broadly classified into exogenous and endogenous Ags.
Exogenous Ags are those that have entered the body from the outside, for example by inhalation, ingestion, or injection.
While endogenous antigens are antigens that have been generated within previously normal cells as a result of normal cell metabolism, or because of viral or intracellular bacterial infection.
Endogenous antigens include xenogenic (heterologous), autologous and idiotypic or allogenic (homologous) antigens.
Ags may also classified as i) T-independent Ags which can directly stimulate the B cells to produce antibody without the requirement for T cell help, in general, polysaccharides are T-independent antigens and ii) T-dependent Ags that do not directly stimulate the production of antibody without the help of T cells. Proteins are T-dependent antigens.
Other classes of Ags include autoantigens and tumor antigens.

Nature of antigens

Antigenic determinants can either be immunogenic or haptenic, the 3 dimensional structures of Ags are important in Ab specificity. It is believed that an immunogen must possess at least determinants to stimulate Abs formation. 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 (ADs recognized by T-cells have 8-15 amino acids). Optical configuration and physical conformation contribute antigenic determinant immunochemical specificity.

IMMUNOTHERAPY

Immunotherapy is also called biologic therapy or biotherapy, it is treatment of disease by inducing, enhancing, or suppressing an immune response, and it incorporates an array of strategies of treatment based upon the concept of modulating the immune system to achieve a prophylactic and or therapeutic goal. Immunotherapy involves the functions of the immune system which includes lymphocytes- B lymphocytes and T lymphocytes that include killer cells, T-helper cells and regulatory (suppressor) cells; and natural killer cells.

There are two main types of immunotherapies;

1. Active- here the body's own immune system is stimulated to fight the disease e.g. cancer vaccines, lymphokine activated killer cell therapy,tumour infiltrating lymphocyte vaccine, interleukine-2 etc

   Vaccines are weakened, killed or live viruses, bacteria and other microorganisms and toxins administered to start an immune response in the body.

2. Passive-use of immune system components created outside the body such as antibodies e.g. monoclonal antibodies, antiserum (*polyclonal antibodies*) etc

   An additional form of immunotherapy is non-specific immunotherapies and adjuvants (immune stimulants) given to boost immune functions and improve how well another therapy works.

MONOCLONAL ANTIBODIES (MAbs)

These are antibodies produced by a single clone of plasma cells having identical structure and specificity and predictability (they bind to only a single Epitope on an Ag). They maybe polymers or monomers or fragments and are also called paraproteins. Normally pure, large quantities of any individual antibody are difficult to produce within an animal, the occurrence of MAbs in serum within the body is mainly due to pathological states called multiple myeloma (a malignant neoplasm of a single clone of plasma cells of the bone marrow that sometimes forms a solitary tumor called plasmacytoma, this often affects synthesis of other clones or plasma cells) or plasmacytoma.

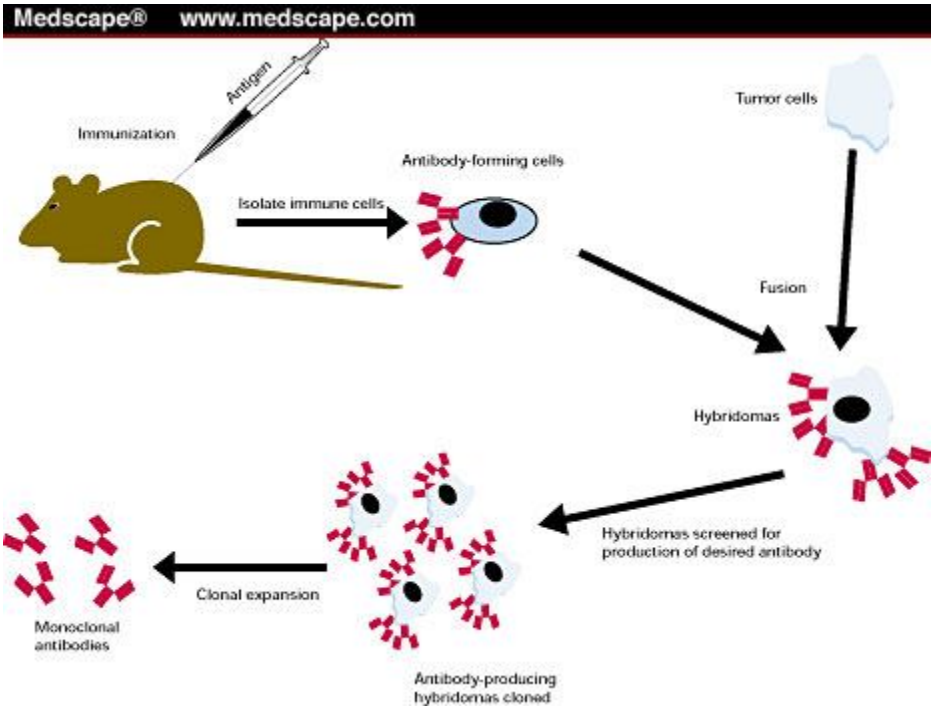
Monoclonal antibodies are the most widely used form of cancer immunotherapy at this time, they also widely used as reagents in immunoassay techniques (diagnosis of diseases).

The method for developing MAbs was developed by Milstein and Köhler and generally referred to as hybridoma technology.

HYBRIDOMA TECHNOLOGY

This involves a series of processes carried out to obtain large amounts of a single clone of Abs specific for one Epitope or antigenic determinant.

- B cells are obtained from the spleen of a laboratory animal that was initially injected with an Ag of interest or mixture of Ags.
- These B cells are fused with mouse myeloma (cancer) cells to make them immortal (otherwise they would eventually die out during propagation in tissue culture) by mixing the two types of cells in the presence of polyethylene glycol (PEG) which causes the cells to fuse by ununderstood mechanisms. The resulting cell is called a *hybridoma*.
- The mouse myeloma cells are cancer cells of the RES, specifically immortal B cells which are deficient in hypoxanthine guanine Phosphoribosyl Transferase (HGPRT) enzyme so cannot synthesize purine bases necessary for production of Abs.
- The cells are then placed in a selective medium- Hypoxanthine Aminopterin Thymine (HAT) medium to grow the fused hybrid cells selectively. The hybridoma cells can survive HAT medium while all unfused cells cannot be maintained in the medium and die.
- The hybrid cells continue to multiply as well as produce Abs. The antibody produced by individual *hybridomas* is characterized (screened, and cell lines detected).Desirable *hybridomas* (i.e., those making antibodies with desirable properties) may be grown and antibody produced via standard tissue culture techniques i.e. cloned in subcultures.
- The hybridoma cells can be frozen and stored and subsequently thawed when more Abs is required. They may also be grown in abdomen of mice and provide large supplies of Abs.



Application of monoclonal bodies (immunotherapy) in medicine and veterinary medicine.

1) MAbs are mainly used in the treatment of cancers. They have important clinical applications in the detection and early diagnosis of cancer
2) They can be used find or identify their specific antigens and this mainly applied for diagnostic purposes e.g. pregnancy diagnosis, disease diagnosis, tentative diagnosis of conditions such as cancer
3) They can be used to measure amounts of individual proteins (measuring protein and drug levels in serum).
4) determine nature of infectious agents (identifying infectious agents)
5) Subclassify both normal and tumor cells
6) Accelerate the removal of drugs from the circulation when they reach toxic levels.
7) Used for typing of T and B cells
8) Detecting serological differences in viruses
9) Experimental treatment of lymphoid malignancies.
10) Monoclonal antibodies are currently utilised in many diagnostic procedures, including:

11) Typing tissue and blood

12) identifying clusters of differentiation for the classification and follow-up therapy of leukaemias and lymphomas

13) identifying tumour antigens and auto-antibodies

14) identifying the specific cells involved in the immune response

15) identifying and quantifying hormones