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Immunogens, Antigens, and Haptens continued.

An immune response can be humoral or cell mediated or both.

Affinity: The measure of strength of interaction between a substance and its receptor.

Avidity: The sum of affinities between multiple molecules of a substance binding with multiples of its receptor. This concept is dealt with in most cases.

The immune response is directly proportional with the strength of the interaction.

Scientifically, immunogens and antigens are different regardless the fact that they are normally used as synonyms. So, immunogens react and produce an immune response and antigens just react with products.

Types of immunogens or antigens:

1. **Allergens**: induce an allergy. They are common environmental substances that cause the production of IgE antibodies. Example are pollen grains, food items, drugs. They generate a special immune response mediated by IgE antibodies.
2. **Mitogens**: substances which can activate a large number of T or B lymphocytes when injected. They have the capacity to generate multiple nonspecific clones of lymphocytes. This polyclonal proliferation leads to the generation of a strong immune response. E.g. lipopolysaccharides (endotoxins of bacteria) are responsible for the polyclonal proliferation of B lymphocytes. Also, hemagglutinin is a mitogen for T lymphocytes. Mitogens are usually used to test the ability of an individual to mount an immune response.
3. **Superantigens:** substances which
   1. are usually derived from bacteria (toxins).
   2. have low molecular weight
   3. need not be processed by antigen presenting cells
   4. can directly link the T cell receptor of the T lymphocyte to class II MHC on the antigen presenting cells without antigen presentation.
   5. can activate multiple clones of T lymphocytes resulting in an enhanced immune response

   e.g. toxic shock syndrome toxin can lead to a severe inflammatory reaction following the polyclonal activation of T lymphocytes.
4. **Tolerogens**: substances that do not evoke an immune response in an individual. All self proteins are tolerogens with respect to our cells. However, this doesn't mean that it's not immunogenic because if it were to

be injected in another species or even a different individual, it may cause a response.

***According to the source of the substance, antigens are:**

1. **Xenoantigens:** derived from different animals of different species. For example, antigens derived from guinea pigs are xenoantigens in relation to humans. Or heart valves taken from pigs and inserted into humans, are also considered xenoantigens.
2. **Heteroantigens:** are antigens shared within different species. Present in humans and pigs at the same time for example.
   Heterophile forssman antigen is a great example for this; it's present in different species, as a consequence heterophile antibodies are produced in the course of infections that can polyclonally activate B lymphocytes.
   The EBV, infecting B lymphocytes, polyclonally activates B lymphocytes producing different antibodies to different antigens. So in human serum, heterophile antibodies act against sheep blood cells without ever been exposed, because they were induced by an antigen shared between sheep and humans (the virus).
3. **Alloantigens**: antigens which are different between individuals of the same species .e.g. antigen A in red blood cells is foreign in a person with the B red blood cell antigen. And the different antigens on white blood cells of different people, is another example.
   In tissue transplantation, antigens are alloantigen unless they are identical or from the same person.
4. **Autoantigens:** are from the same host.

Haptens:

- The only thing that prevents inducement of an immune response by haptens is SIZE.
- Haptens are antigens but NOT immunogens.
- Haptenic utilization of certain substance is one of the ways the body mounts responses... sometimes to self proteins and autoimmunity can result from this phenomenon.

  For example, autoimmune haemolytic anaemia in response to the administration of certain drugs develops when injecting a drug, which will adsorb to red blood cells. The carrier and hapten are recognised as foreign substances, the three types of antibodies will develop. Since the carrier is the red blood cell, this will lead to haemolysis. Methyldopa is known for being associated with autoimmune haemolytic anaemia.

Factors are requirements for immunogenicity.

1. **Contribution of the immunogen**

a. Foreignness is the single most important factor (condition) in determining the immunogenicity of any substance; any other factor could be modified. Self-antigens –usually- cause no immune response.

b. Molecular Weight:
-An important requirement.
-Less than 1000 Daltons are haptens e.g. drugs, which are too small to cause an immune response.
-1000-6000 Daltons: may or may not be immunogenic, this depends on other factors such as: nature, complexity, method of administration...
-Small molecules can be modified in order to become immunogenic, like increasing their complexity, or adding a carrier...
-The higher the molecular weight the stonger an immunogen is.

c. Chemical Nature and Complexity:
-Proteins are the most immunogenic, then carbohydrates, then nucleic acids then lipids.
-Heteropolymers are more immunogenic than homopolymers.
-Aromatic amino acids in proteins are more immunogenic than nonaromatic.
-The more complex the structure, the more the response.
-Primary then secondary, then tertiary then quaternary structures of proteins are ordered in <u>increasing</u> immunogenicity.

d. The presence of an antigenic group:
Because the B or T lymphocyte receptor is not capable of accommodating the whole protein and dealing with it as an antigen, only small and certain segments of that protein or carbohydrate (few amino acids or sugars) are involved in the immune response. The binding only involves these segments. These segments are named **epitopes**, they are also called antigenic determinants.
The part of the T cell or the antibody receptor is known as the **Paratope.**

e. Physical Form of the substance:

-Particulate, insoluble > Soluble: As red blood cells are very soluble, they mount a very strong and fatal immune response when blood is transferred from one person to another.

- Denatured > native: even if they are self antigens. For example, albumin is non immunogenic, however, when it undergoes ultracentrifuge (becoming denatured), it will mount an immune response.

f. Degradability: presentation of the antigen (needed for action of both T cells and B cells to recognise the antigen) requires for the antigen to be degraded, or digested by macrophages.

2. **Contribution of the Biological System**

a. Genetics:

At the level of individuals of the same species, the difference in immune responses is related to the immune response genes in each host. The MHC genes on a locus on the short arm of chromosome number six, determines the ability of an individual to mount an immune response or not.
E.g. when a vaccine -let's say hepatitis B, is given to a population, 2%-5% will be non responders and will never mount a response. For any vaccine, regardless the times shots are repeated, 100% responsiveness is never present.

b. Age:
-Immune competence deteriorates with age.
-Neonates and the elderly do not mount a proper immune response. As said before, Hepatitis B establishes chronicity in 70%-90% of neonates because they fail to mount an immune response that can eliminate the virus. Vaccines are delayed till the third month of the newborn's life for the same reason.
-Before the age of two, neonates can never respond to polysaccharides. This is why Streptococcus pneumoniae and Haemophilus influenzae are very dangerous infections because of the failure to respond to the polysaccharides of the protective capsule of both. Their vaccine (the Hib vaccine) is made my coupling these antigenic polysaccharides to protein carriers.

3. Method of Administration

There are three interrelated factors:

a. Dosage: for any substance there is an optimal range. Anything less or more will NOT induce an immune response. In other words, extremes in the dose are tolerogens.
b. Route: Subcutaneous >I.M> I.V > oral (because of the presence of mechanisms that establish tolerance: "Oral Tolerance" as said previously.)
c. Rate: the order above is so because the route is related to the rate of elimination. The fastest rate of elimination is the I.V depriving the immune system from the chance of dealing with this injected antigen.

From this comes the concept "adjuvant". The most used mechanism for enhancing the immune response is decreasing the rate of the release of the antigen thereby allowing for its persistent stay in the body.

They differ from carriers in that they <u>enhance</u> the immune response of IMMUNOGENS ONLY.

Freund's incomplete adjuvant is composed of an oil and water emulsion, decreasing the rate of elimination. Adding TB mycobacteria to it will give the complete adjuvant that activates T lymphocytes. Alum precipitate is the most used in vaccines.

Cross reactivity

Substances may be similar; this structural similarity is referred to as "cross reactivity".

Products of the immune system against these similar substances can cross react. The structural similarity could be high, moderate or low and the more the similarity the more likely for a cross reaction to take place.

***This is really important in:

1-**Autoimmunity**:

One example is that Streptococci are responsible for rheumatic fever; the antibodies against the bacterial antigens can cross react with an antigen present in heart valves. These fail to recognise the difference between the two types of antigens because of the strong similarity.

Autoimmunity associated with cross reactivity is also believed to be related to rheumatoid arthritis, SLE (Systemic lupus erythematosus), diabetes and other diseases which also occur after certain infections.

2- Another significance of cross reactivity is the **ABO system**, the blood groups. Individuals of the A group have in their serum anti-A antibodies while group B individuals have in their serum anti-B antibodies. Group O have both anti-A and anti-B. Those with the AB subtype have none.

Where did these antibodies come from as individuals are not exposed to the RBC antigens? It is believed that the source of stimulation for the production of these antibodies is microbial (bacterial) antigens.

Individuals are born without these antibodies; neonates do not have anti-blood group antibodies. They start to develop these antibodies as they grow. Bacteria are established as normal body flora in them. Such bacteria have capsules that are composed of polysaccharides, theses polysaccharides are similar to the polysaccharides that make up the antigens of the red blood cells. And that is how we develop antibodies to RBC antigens that we were never exposed to. These antibodies develop and are present in children by the age of one year. And these must have a titer of 4 minimum. Titer means the highest dilution which still gives a positive reaction, meaning if we take the serum, of a child one year of age and dilute his serum 4 times it must still react with RBCs, if the child lacks these antibodies by the age of one year then this is an indication of immunodeficiency. Humoral immunity is assessed by the measurement of these antibodies, which are called: "**isohemagglutinins**".

So cross reactivity between antigens is important, it can be involved in autoimmunity, in tolerance and the production of antibodies to the RBC antigens.

Antigens are either T- dependent or T-independent meaning they require the T cell help or don't require their help.

- Those that are T-independent are polysaccharide in nature and are polymeric in structure. They activate B lymphocytes polyclonally and they don't cause generation of memory cells.
- T- dependant antigens have the most multiple antigenic determinants of different features.

Hapten-carrier conjugates

Proteins can be modified by haptens; this can be done in vitro or in vivo as mentioned previously of drugs that are utilized by an RBC or protein to create haptens.

Epitopes may be linear or discontinuous (conformational).

- Discontinuous epitopes are formed by conformational changes that bring them close to each other.
- Linear epitopes are present next to each other.

Antigenic Determinants Recognized by B cells and Ab and T cells

- **B cells can recognize all antigenic determinants**. The size of antigenic determinants recognized by B cells are small, they may be 4-8 amino acids or 4-8 sugar molecules. They are limited in number by the immune-dominant epitopes present on these surfaces and they can be present on the external surface of the antigens.

In the slide, the folding of the protein creates a structure which is discontinuous or conformational. These are brought together as a consequence of the conformational change of the protein and are recognized by B cells.

- **Antigenic Determinants Recognized by T cells** recognize sequence determinants that are processed by macrophages. T cells do not recognize conformational epitopes because t cells only recognize processed antigens which are presented in sequence, in a linear from. B cells can deal with unprocessed antigens as they are themselves antigen processing cells; that is why they recognize conformational epitopes.

The size of an epitope recognized by T cells is double the size recognized by B cells. So T cell receptors may accommodate larger sequences of an epitope but the number of epitopes that can be dealt with is dependent on the number that can bind to an MHC complex.

Superantigens

- Normally T cells recognize antigens that are presented by an antigen presenting cell via the MHC molecule, the superantigen can bridge the MHC molecule to the TCR without the requirement of processing or presentation in conjunction with an MHC. It can in itself bind the MHC to the TCR and stimulate the activation and proliferation of the T cell.
- The importance of superantigens is illustrated in the slide. If you inject an individual with a protein you can maximally stimulate one out of 10,000-100,000 lymphocytes. The rate of T cells that can be activated is 10^{-4}-10^{-5} maximum, whereas the superantigen can activate 10-25% of the T lymphocytes. This makes the superantigens much more potent. That is why in toxic shock syndrome, individuals develop severe systemic disease and they die sometimes within 24 hrs as a result of the activation of this huge number of T cells and the resulting inflammatory response. They develop severe inflammation, shock then death.