Immunology 8

Immunoglobulins properties

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**Those are just extra notes for “Immunoglobulins properties” slides

Introduction:

Immunoglobulins have protective functions which enable the living organism to fight multiple different infections.

Neutralization of toxins : whether bacterial toxins, or toxins of reptiles and insects such as venoms, snake and scorpion toxins.

Immobilization of organisms: antibodies can fix organisms by interacting with the organism as a whole or with flagella (organs that are essential for micro-organism movement)

Neutralization of viral infectivity : can be a result of blocking the ability of the virus to attach (blocking the attachment site of the virus for the receptor) or interaction with virus; blocking a later phase in reproduction of the virus like; penetration, transcription and coating of the virus.

Agglutination of microorganism = tamping of micro-organism

Binding with soluble antigens: antigens can be soluble like: bacterial products, microbial products and foreign proteins. Those antigens make the body produce antibodies that combine with the antigens to produce immune complexes, and those complexes bind to phagocytic cells that causes the clearness of this antigens (if the antigens here not cleared it will remain in the circulation and continue to stimulate the immune response which may be harmful to the body).

Activation of complement is the function of antibodies either in the classical pathway (activated by antigen antibody complexes) or alternative pathway (activated by aggregated immunoglobulins). Complement system is essential for phagocytosis and to mount an inflammatory response that are essential for the completion of the function of immune response.

Protection of fetus: is achieved by antibodies - coming from the maternal circulation - that cross the placenta and provide protection from the organisms that the mother is immune. If the mother is not immune to a certain organism then those organisms maybe harmful to the fetus.
Immunoglobulin Structure-Function Relationship:

Immunoglobulins can be either attached to B-cells and work as a receptor for antigen or it can be secreted.

Immunoglobulins are Bifunctional Proteins:

Immunoglobulins can interact with a small number of specialized molecule (above mentioned) through its Fc fragment (that has very small variation), on the other hand they can interact with infinite numbers of antigens (as antigens interact with Fab which carries a huge variability).

Why do antibodies need an Fc region? :

Precipitate the antigen through binding to it.

Block the active sites of toxins or pathogen associated molecules that neutralize infectivity or the effect of the toxin.

Why do antibodies need an Fc region? (cont.):

Such functions require the Fc fragment as those functions are complementary to antigen binding (e.g phagocytic function and all the above mentioned functions).

Four distinct roles of Fc binding proteins:

1- it happens through IgA that is synthesized by intraepithelial lymphocytes then it is bound to poly IgR that allow dimeric IgA to cross epithelium into the luminal side of the mucus membrane, also pentameric IgM cross in a similar process.

2- FcRN: is a receptor on placenta that binds to IgG and allow it to cross the placenta, without such binding IgG can’t cross the placenta.

** it was thought that IgG can cross the placenta due its molecular weight but IgE, IgD and IgA have similar molecular weight but don’t cross the placenta ,so it was discovered that the ability of IgG to cross the placenta is due to the structure of Fc fragment of IgG.

3- Fc is required to trigger effector functions; as IgG binds to its Fc receptor on macrophages and so it is an opsonin , it also has a receptor on natural killer cells (NK) and that is how NK kill by ADCC ( antibody dependant cell-mediated cytotoxicity).

4-Cross-linking of FcR which generates immunoregulatory signals that affect cell activation, differentiation: IgE is present on basophiles and mast cells and its binding to those cells is the first step in initiating an allergic reaction as allergen binds to IgE which is present on those cells. Similarly the Fc fragment of immunoglobulin on B-lymphoctes is essintial to create the signal
that will initiate a response to cascade the events leading to the activation of B-lymphocytes; cross-linking of B-cell receptor by an antigen is essential to create a signal on B-lymphocytes.

**The structure of a number of human Fc receptors:**

Fc fragment has an important role and it does its role via a receptor; that is usually high affinity receptor. Those different receptors are found on the surface of different cells each for a different kind of immunoglobulin (know the different kinds of receptor that are in the figure).

**Biological Properties of IgG:**

It is the most important immunoglobulin.

Most abundant immunoglobulin in serum up to (70%-80%) of immunoglobulin.

Distributed equally between the intravascular and extravascular spaces so it is present in the CSF, serum, peritoneal fluid, GI fat, GIT fluid. You can find IgG in all fluids.

As IgG has the longest half-life, it is the most abundant.

When IgG is produced in high concentration it remain for a long time in the serum as in the cases of measles, yellow fever and hepatitis A the concentration of IgG produced is very huge that they remain in the serum for life-time.

IgG production is associated with generation of memory B-lymphocytes that can produce IgG in secondary immune response which maintains IgG in the serum of normal individual.

About the 4th point: it is a mechanism that the body uses in order to avoid high concentration of immunoglobulin. There is always a ratio between albumins and globulins and this ratio is imbalanced in the case of high production of immunoglobulin causing hypergammaglobulinemia favoring one reactant over the other causing the increase of the rate of catabolism of immunoglobulins.

**Functions of IgG (10-12):**

There are two major types of antigen antibody reaction: agglutination, and precipitation. The reaction that results depends on the solubility of the reactant and both reactant solubility can vary as the antigen can be insoluble (e.g. bacteria) and the antibody can be also insoluble (all immunoglobulins are soluble in serum but IgM and IgD can be insoluble if they attached to B-cells) so we can have three cases (the doctor just mentioned two of them):
1- both reactants are soluble --- the reaction is precipitation

Precipitation is the formation of precipitate of large complexes as the result of interacting soluble antibodies with soluble antigen; IgG is very efficient as antigen-antibody reactant making precipitate when the antigen is soluble actually. Actually precipitate can be in vitro as in the body the complexes that are formed are removed by phagocytic cells.

2- if the antigen is insoluble (e.g. bacteria, erythrocytes) and the antibody is soluble the result reaction is agglutination (as IgG clamps the antigens; bring them together)

As we know that IgG has just two Fab (2 antigen binding sites) and it is relatively short so it may not be able to bind large number of cells so the agglutination maybe invisible because of size limit (although agglutination is a visible reaction) and we can make it visible by adding anti-IgG so the reaction will become larger.

IgG2 has limited ability or doesn’t go through the placenta.

The passage of IgG through the placenta can beneficial for the baby as to immunize it against the diseases that the mother is immune to, on the other hand it can cause a disease called hemolytic disease of newborn or Erythroblastosis fetalis; as the mother can have a different blood groups than the fetus the most important blood group that can be different is: the Rh; is composed of six antigens –in wiki 5 antigens- they are C, c, E, e, D (d indicates negative for D antigen and it is used to indicate that the individual has negative Rh). If the mother is negative for Rh and the fetus is positive, transplacental hemorrhage may result from the exposure of fetus blood to the mother’s early in pregnancy or the mother could have synthesized anti-Rh from a previous pregnancy, previous abortion or even blood transfusion. So as a consequence of this sensitization, in the next pregnancy, transplacental hemorrhage will result in the production of large amounts of IgGs, and this is a secondary immune response. Those IgGs will pass through the placenta and reach the blood of the fetus and cause “hemolytic disease of the newborn” and this will result in what is called hydrops fetalis in which the baby will die in the uterus because of severe hemolysis. Sometimes we do intrauterine blood exchange in order to save the fetus.

About opsonizations: there are two opsonins; IgG and C3b.

-ADCC: Antibody dependent cell-mediated cytotoxicity. It is a killing mechanism mediated by antibodies by natural killer cells.

-Activation of the complement is achieved by IgG as being part of an immune complex (Ag-Ab complex) and this is the classical pathway, or aggregated IgGs and this is the alternative pathway.
- Neutralization of the toxins: IgG is the BEST to neutralize the active site of the toxin.

The snake venom can cause severe hemolysis, and thus severe hemolytic anemia.

**Immobilization of Bacteria** either as a whole or by the reaction with the flagella, so the bacteria will be agglutinated by IgG.

**Important Differences Between IgG Subclasses:**

IgG is the most abundant immunoglobulin.

The most important and the most abundant type of IgGs is IgG1, and it performs all functions with a half-life of 23 days.

Other subtypes are less important and differ from each other.

IgG2 differ that it is unable to cross the placenta.

IgG3 differ that it has a short half-life, it doesn’t really affect the total IgG half-life as it has a low concentration.

IgG4 does not bind to the complement or to monocytes.

**IgA dimerisation and secretion:**

IgA as a serum immunoglobulin has no important function. Actually the function of serum immunoglobulins are limited to IgM and IgG (IgM is the first to be produced in an immune response then comes the IgG then the IgA that is produced at a later phase). But IgG is very important in secretions it is the only arm of humeral-mucosal immunity (humeral immune response at the mucosal surface) but there are also short comings for mucosal immunity mediated by IgA because of the transient nature of its protection, as protection provided by IgA antibodies lasts for about 6 months to 2 years only, and it acts on the respiratory tract, GIT as well as the genitourinary tract. Whereas serum protection can last for decades, so the protection provided by IgG can last for several years or decades.

*IgG is responsible for serum protection

IgA1 is the most abundant both in serum and secretions.

**Secretory IgA and transcytosis:**

The secretory IgA is produced from intraepithelial B cells -that are located in the submucosa-they produces dimers of IgA, then this dimer binds to poly IgR and both; the receptor and IgA
are internalized by the epithelial cells, which adds the secretary piece to the immunoglobulin and secrete it to the lumen of the mucosa where it provides protection.

Properties of IgA:

It is bactericidal for Gram negative bacteria in the presence of lysozyme and not alone.

Agglutinating activity of insoluble agents.

IgA facts and figures:

There is no complement activation by classical pathway but by alternative pathway (only IgA1).

IgA interacts with the epithelial cells by the Polymeric immunoglobulin receptor \([\text{pIgR}]\), and interacts with the phagocytic cells by the IgA receptor.

IgA is inefficient at causing inflammation because it doesn’t activate the complement or other cells.

Vulnerability: susceptibility

IgA1 can’t be truncated because the hinge is heavily glycosylated.

Biologic Properties of IgM:

- IgM is the first to be produced in the immune response.

- IgM is NOT present extravascularly.

- It is the only immunoglobulin class synthesized by the fetus beginning at approximately 5 months of gestation >> this fact is very important because it enables the diagnosis of the intrauterine infections like rubella ... etc.

If the fetus has immunoglobulins of IgG class we cannot distinguish if it is from a maternal or a fetal source unless we wait for a while and measure the concentration again (if it is maternal then the concentration will decline, but if it is fetal then the concentration will rise).

But if the baby has IgM it is for sure an intrauterine infection.

- The only bacterial infection that crosses the placenta is syphilis, and the only parasitic infection is toxoplasmosis, and the remaining are viruses (many viruses can cause intrauterine infection).
Functions of IgM:

- IgM is the most efficient one in agglutination because it has 10 binding sites (they actually can bind just 5 antigens at a time)

- Isohemagglutinins: are antibodies directed against red blood cells’ antigens, they are usually absent at birth but start to appear by the end of the first year of life (these antibodies normally reach the titer of 4), they are against the blood group antigen that the individual lack. They are acquired by cross-reactivity with bacterial antigens. An individual will have antibodies against the blood groups that he doesn't have (even if he was infected with a bacteria that has cross-reactive antigens with his RBCs’ antigens the body will recognize it as self so no AB will be produced)

- The creation of blood group antigens is a function of transferase enzymes that we inherit; N-acetylgalactosaminytransferase and galactosyltransferase. This enzyme transfers the sugar and adds it to the red blood cells, so the addition of the sugar will create a protein.

The addition of galactose creates the B protein, and the addition of N-acetylgalactosamine creates the A protein. The addition of both creates the AB proteins. If there is no addition, the result will be the O blood group.

Activation of complement by IgM by the classical pathway.

Polymeric IgM:

Polymeric are formed in secreted immunoglobulins.

Cm4 mediates multimerisation of immunoglobulins.

Multimerisation of IgM:

It is the secretory piece or a membrane segment that determine if the IgM will be a pentamer or a monomer (this will be discussed later in immunoglobulin diversity).

Biological Properties of IgD & IgE:

IgD activate B-cells.

IgD facts and figures:

IgD may react with T-cells but it is most abundant on naïve B-cells.
IgE facts and figures:

Very low concentration in serum

Role for IgE on mast cells and basophils:

IgE binds to Fc receptor on mast cells, as the allergen binds to IgE attached to FcR, this cross linking of IgE will create a signal that transfer via the cell membrane and works on the expulsion of granules of the mast cells and the release of its contents.

Sero-therapy:

This mechanism can be used in patients who are sick of tetanus or diphtheria.

Passive Immunity:

Passive immunity can be natural (like mother to fetus) or artificial (like injecting immunoglobulins to the host).

Antibody therapy:

When the host is immunodeficient and can't make its own antibodies we inject him with large concentration of immunoglobulins with wide specificities. So that those individuals can live a normal life by getting antibodies from others.

The conventional polyclonal antiserum contains a mixture of monoclonal antibodies:

Production of antibodies can be polyclonal or monoclonal. Polyclonal can be produced in your body as multiple B-lymphocytes are involved.

Monoclonal antibody and Hybridoma:

We can select a single B-lymphocyte by producing single cell suspension, fusing them with tumor cells and produce more and more antibodies.

The above figures shows the technique of last century to produce monoclonal antibodies; that is by using animals and creating of hybridoma (fusing multiple myeloma cell with normal B-lymphocyte to create immortal cells that can produce infinite number of antibodies)

Uses of Monoclonal Antibodies:

In diagnosis we use them to target a single molecule like CD5, CD3 on T-lymphocytes to identify them
Therapeutic agent as in cases of cancer we can direct monoclonal antibody to antigens on cancer cells or we can add to this antibody a toxin or any other chemotherapeutic agent so the antibodies will act as a poison arrow that target very specific structure on the cancer cell that causes the killing of the tumor cell.

**CD52 is strongly expressed on lymphocytes and not on blood stem cells:**

We can eliminate a certain type of cells from a population like eliminating lymphocytes from blood as CD52 is strongly expressed on lymphocytes and not on other kind of blood cells so we can use anti-CD25 with complement that will lyse all lymphocytes.

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