Tipos de precipitacion inmunologia

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Immunological methods for studying the functionality of phagocytes Page 22 2. 1 1. ANTIGEN-ANTIBODY INTERACTION Antigen-Antibody Binding (Ag-Ac) is a reversible interaction involving non-covalent connections. In the test tube, the interaction of the antigen (Ag) with the corresponding antibody (Ac) two stages are different, the primary non-visible interaction and secondary interaction, which follows the previous and is characterized by the appearance of a visible phenomenon, such as agglutination or precipitation. On the other hand, the initial interaction of Ag-Ac does not always occur, there is a secondary interaction, because to achieve visible phenomena certain concentrations and characteristics of Ags and Acs are required. 2. SECONDARY INTERACTION OF ANTIGEN-ANTIBODY. Immune methods that demonstrate ag-ac interaction through secondary response (precipitation or agglutination) are generally cheaper and easier than those that allow visualizing primary interaction (see below). Second. Reaction precipitation By mixing enough soluble Ag with specific Acs, Ag-Ac interaction can lead to a network capable of visualizing as sediment. This network we are talking about is nothing more than large immune complexes (I's) formed by Ag-Ac interactions. As shown in the figure, as the concentrations of soluble Ag are added to a fixed amount of serum containing specific Acs, as the amount of Ag added increases, the amount of precipitation increases to the maximum, after which it decreases. At one end of the precipitation curve, when small amounts of Ag are added, I's formed over Ac. On the other hand, by adding a large amount of Ag, I'm formed over Ag to be a small probably consists of one Ac molecule and two Ag. Between these two extreme situations is an equivalency zone where the Ag-Ac ratio allows you to create large I networks that are deposited. The reaction of the rash depends on VALENCIA Ac and Ag. Ac Valencia gives an idea of the number of sites it owns to recognize Ag. Thus, bivalent Ac has two sites capable of recognizing Ag. Similarly, Ag's valence tells us about the maximum number of epitopes he possesses. In order for ag-Ag secondary interaction with subsequent precipitation formation occurs, both Ag and Ac must be at least bivalent. Excess Ac Offset Excess Ag Ag Ag Number added 3. 2 2.b. Immunological methods based on precipitation reactions. 1- Radial immuno diffusion (IDR) This method allows a simple and economical quantitative assessment of isotype G, M and A immunoglobulins or soluble antigens (C3, C4, transferrin, etc.) present in biological samples. The technique is based on the diffusion of a sample containing an antigen that will be measured (immunoglobulins or soluble antigens) in a semi-solid agar matrix in which a specific ac dissolves. The biological sample is inserted into the cylindrical holes dug in the agar, and as the antigen dissipates radially, the Ag-Ac ratio is achieved, allowing visible precipitation formation. After the completion of diffusion, the radius (R) of precipitation is measured, which is proportional to the concentration of antigen (C). The concentration of protein in the biological sample (Cx) is calculated by performing a calibration curve using known samples of the concentration structure. 2- Immunoelectrophoresis (MEF). Immunoelectrophoresis is a qualitative method that allows to identify various protein components present in different biological samples (whey, urine, etc.) through the arcs of precipitation. The technique is preferably performed on agaro gels and the two stages are different: 1) Samples undergo electrophoretic separation. The connection between the ac and Ag bivalent monovalents (one non-repeated epitope) does not allow the formation of a besieging I. The connection between bivalent Ac and Ag multivalent allows the formation of the besieging I. Multivalent Ag may have one repeated epitope or several different epitopes. Rx R1 R2 R2 R3 C3 C3 Biological sample of unknown concentration (Cx) C1 C2 Sample of the known concentration model (C) R 2 C1 C2 Cx Rx 2 Halo 4 sediment. 3 2) After electrophoresis, the proteins interact with the specific acs applied in the agar in the channel parallel to the migration axis. After the Acs diffusion period, there are arcs of precipitation, the shape and position of which depend on immunochemical characteristics and concentration Protein. 2.c. Agglutination reaction agglutination reaction, as we have seen for precipitation response, involve a secondary interaction between Ag-Ac, which will lead to the appearance of a binder that is visualized as lumps. The physical principles that regulate the formation of these agglutinates are the same ones that regulate the formation of sediment. In other words, the principles mentioned above are about the area of excess antigen, the equivalency zone and the zone of excess antibody are valid and applicable to the agglutination of reactions. The big difference between precipitation reactions and agglutination reactions is the characteristics of the antigen: while soluble antigens are used in precipitation reactions, Ag is a binder of particulate matter. With soluble Ag, it is also possible to develop an agglutination reaction because it is possible to use different particles (inert particles or red blood cells) and perform chemical or physical adhesive soluble Ag to these particles, resulting in useful particles for diagnostic purposes. The advantages of agglutination reactions in terms of their usefulness in the lab is that they are very easy to perform, do not require any equipment to read, are fast and easy to implement. In addition, they have a higher sensitivity than precipitation reactions so 5. 4 replaced in many cases. However, it should be noted that agglutination reactions have lower sensitivity than primary interaction reactions such as ELISA and indirect immunofluorescence (see below). This means that in some cases it is preferable to use one of these two methods to detect Acs against a particular pathogen. These benefits make reaction agglutination a valuable tool for various serological studies (search for specific Acs against pathogens) and for detecting antigens in bodily fluids. Types of agglutination reactions according to the characteristics of binders: According to the characteristics of the particle binder, agglutination of the reaction can be classified as: - active agglutination reactions; o - passive agglutination reactions. Active reaction agglutinations are based on the use of a binder particle as an antigen. Examples include: Particle Swallowing Reaction Diagnostic Utility Red Blood Cells Group Definition and Rh Factor Red Blood Cells Indirect Coombs Incompatibility Definition Rh Red Blood Cells Coo Direct Erythroblastoization Bacteria (Brucella) Haddlason Search ac anti-Brucella in Human Serum (Blood Bank) Parasites (Tripanosoma Cruz) cruzi in human serums (blood bank) Parasites (Toxoplasma gondii) AD-Toxo Search Ac anti-T. gondii in human serums (blood bank) Passive reaction agglutination is based on the use of an inert binder particle to which the antigen attached it. As examples we can mention: Particle Swallowing Reaction Diagnostic Utility Red Blood Cells - T. Krusi HAI-Chagas Lysate Search Ac Anti-T. Cruzy in Human Serum (Blood Bank) Red Blood Cells - T. gondii HAI-Toxo, Licking Search Ac anti-T. Gondy in human serums (blood bank) Latex and gp120 Search ac anti-HIV in human serums (blood bank) latex and sHBV Search Ac anti-HBV in human serums (blood bank) latex and streptolisin or ASTO Search Ac anti-streptolisin O in human serums (streptococcus) solid particles supports for indirect agglutination reactions may be red blood cells (in this case hemagglutination) or latex particles is usually said. The use of this type of inert particles has expanded the usefulness of the agglutination method to the definition of Ac against infectious agents such as HIV or HBV, and even to identify Ac against soluble molecules (strepolisin O, C-reactive protein, etc.). As an indispensable step for the preparation of this type of reagents, the incubation stage between the inert particle and the sensitizing soluble antigen (in general, Ag sensitization are proteins, although carbohydrates can be immobilized), allowing the particle to be glued to the particle. This procedure can be performed by a simple adorption (non-covalent binding of Ag to a particle) or covalent binding. In most cases, a precondition for particle surface is required. For red blood cells, this is achieved by incubation with bloumic acid (a process called tanado) or with CrCl3. Once Ag is glued to the surface of erythema blood cells, the particles can be corrected with chemical agents such as glutaraldehyde to give them 6. 5 increases stability over time and changes in temperature, increasing the life of the diagnostic reagent. Types of passive agglutination reactions depending on the identity of the species glued to the binders: While it is possible to immobilize many different types of proteins to different types of inert particles, it is possible to classify passive react agglutination in: - direct passive agglutination reactions; o - reverse passive agglutination reactions. Direct reactalizations are based on inert particle sensitization with Ag, so they are useful for detecting and tingrating Ac in various bodily fluids. As examples, it is enough to consider In the table above. Conversely, reverse react agglutinations are based on the immobilization of Ac (generally, monoclonal antibodies - AcMo-) immobilized on the surface of the inert particle, such reactions are especially useful for detecting Ag in various biological samples. 2.d. TITLE concept has already been mentioned that direct agglutination reactions are useful for detecting and tingrating Ac in various bodily fluids. It is important to note that it is impossible to determine ac concentration, but at best, it will be possible to calculate the degree of Ac. To do this, the serial dilution of the patient's serum with a constant amount of Ag is incubated and the name is arbitrarily selected to reverse the maximum dilution of the serum, which produces visible agglutination. In some samples, where there is a large amount of air conditioning, it is possible that at low dilutions (i.e. at high concentrations of serum) agglutination is not observed. This phenomenon is called the prozone effect and occurs because more soluble Ac Ag-Ac complexes (such as those that form in the excess Ac in the precipitation curve) are preferably formed. In this case, the name ac remains the maximum dilution of the serum, which produces visible agglutination. Ag Particles: Permanent Serum Amount: AGLUTINACION Dilution Increase 1/2 1/4 1/8 1/16 1/32 1/64 - - - TITLE: 32 Prozonaicula Inerte Soluble Ag ReVERSA Direct Passive Agglutination 7. 6 With two serum samples from the same patient taken at different times, the name Acs can be compared to this method in both samples. SEROCONVERSION is called the appearance of acs or an increase in the name acs 4 times between 3 and 4 weeks. In the case of reverse agglutinations, it will be possible to calculate Ag concentration in the sample if the titration curve is performed in parallel with the using the standard Ag solution. For comparison of the maximum dilution of the standard solution, which produces a visible agglutination with maximum dilution of the sample producing visible agglutination, it is possible to calculate the concentration of Ag in the sample. There is also an agglutination option that allows you to calculate Ag concentration in the sample. This method is called hemagglutination inhibition and consists of incubation of a constant number of agglutinating particles sensitized with Ag Of Interest (Ag particles), with permanent and binding amounts of a particular Ac. This bind-to-binding blend collides with a variable amount of Free Ag as a competitor. From a particular concentration of the free Ag, there is an ag libre inhibition of the competition ag ac (which thus will not join the Ag particle to bind). If such a curve is made with The standard free Ag solution can be calculated by concentration (micromolar, g/ml, etc.), which suppresses agglutination. Then, by determining what dilution of free Ag is present in the biological sample produces such agglutination inhibition, it will be possible to calculate the concentration of free Ag in the problem sample. Detection of IgG and IgM'IgG: Agglutination reactions with and without 2-mercaptoethanol (2-ME): Several diagnostic reagents are currently available on the market and in laboratories allow to determine whether ac name received in direct or indirect agglutination response corresponds to IgG or the amount of IgM plus IgG. This is because both types of immunoglobulins binder. On the other hand, in many cases it is helpful to know if the patient is IgM or IgG type Ac, because it can facilitate the patient's follow-up or determine treatment. To do this, the agglutination technique was developed in the absence and presence of 2-ME. When the patient's serum titration is performed by agglutination in the absence of 2-ME and titration is reported, in fact the binder activity is reported to be the amount of mandatory activity of IgM and IgG. If, following the protocol provided by the manufacturer, the equipment is performed in the presence of 2-ME produces a smooth/partial reduction of IgM. Since the received monomeric IgM is not a link, the name obtained during agglutination in the presence of 2-ME should be consistent with IgG's exceptionally binding activities. Thus, the implementation of agglutination in the presence and absence of 2-ME will determine the presence of IgG and IgM against Ag sensitization. In some cases (toxoplasmosis) it is important to determine the presence of IgM, because it is a sign of an active infection. The fall of the serum name by agglutination in the absence of an anti-in-the-presence 2-ME indicates the presence of specific IgM for the parasite. Red Globulo with Ag and Ac (connector ratio) AGGLUTINATION - - - - - Inhibiting hemagglutination without free Ag Free Ag: Increased dilution 1/2 1/4 1/8 1/16 1/32 8. 7 3. PRIMARY INTERACTION ANTIGEN-ANTIBODY Although the initial Ag-Ac interaction is not visible, there are various methods that make it possible to visualize it. The strategy is to label ac or Ag with covalent binding (conjugation) of certain molecules, such as FLUOROCROMOS, RADIOACTIVE ISOTOPOS or ENSIMAS, in order to make this first interaction visible. As you can see from the table, depending on the marker used, the immune method is given a certain name and uses a different detection system. The methods we will see below are not as simple and economical as the ones we have seen above, however they are much more sensitive, i.e. they are able to detect lower concentrations of Ag or Ac. MARKER Detection System Enzyme optical microscope Fluorochromic fluorescent microscope Flow cytometer b- Radioimmunoanalysis (RIA) Radioimmunometry methods (PRIST, RAST) Radioactive isotope Radiation counter c- ELISA Enzyme Spectrophotometer 3rd immunofotometer Immunomarkation Immunomarkation Immunomarkation techniques using conjugate Ac allow to detect the presence of ags in tissues (immunohymia) Tissue marking is performed on the cuts of fixed tissue installed on the slide. With these methods, it is possible to detect both cellular (membrane, cytoplasmic or nuclear) and extracellular (extracellular matrix, basal membrane, etc.) cell imunomarkation can be performed on live or fixed cells that can be suspended or attached to solid support (slides). This method allows the detection of Ags located in the plasma membrane, cytoplasm or nucleus of the cell. DIRECT immuno-tagging is the easiest way to find Ag and includes the use of a specific marked Ac for this Ag (Primary Ac). To perform this technique, the sample is incubated by the primary ac, marked over a certain time, at a specified temperature, after which the former Ac is removed by washing and detection sign. THE INDIRECT immuno-tagging uses an unmarked primary ac, which recognizes Ag's interest, and then, to show the presence of Ag, uses a second marked Ac (Secondary Ac) capable of recognizing the primary Ac. For example, if the main Ac is the IgG mouse, the secondary Ac may be a rabbit made by a mouse anti-IgG. DIRECT IMMUNOMARCATION Ac is marked Ag Cell Slides 9. 8 To perform this technique the sample is incubated with the primary ac, as we have seen above, the wash is performed to remove the ex-ac and then incubated with the second marked Ac. Subsequently, the washing is carried out and the signs are checked. IMMUNOmarking ADVANTAGES ADVANTAGES Direct Fast and Simple. To detect each Ag requires primary ac (marked AEs are more expensive than unmarked AEs. Less sensitive. Indirect more sensitive. As immuno-marking (direct or indirect) has been performed, the presence of Ac conjuged with an enzyme is evidenced by the addition of a colorless substrate. The effect of the enzyme on the substrate generates a color product that precipitates in the place where the reaction occurred and is visualized under an optical microscope. Colorless substrates that vibrate in a color product are known as chromogens. The fact that there are various chromogens that can generate Color, allows a different Ag to be detected in the same cut of tissue or cell. Immunomarking with AK chained to FLUOROCROMOS This method is widely known as immunofluorescence, used for both imnunohsthostimimia and imnunocytochimimia. The presence of Ac conjugated with flourochrome does not need any chemical reaction to become apparent. Fluorchromes emit light of a certain wavelength after being hit by a smaller beam of light wavelength. Each lyrochrome is capable of emitting light within a certain wavelength spectrum. For example, a fluororichra called fluoreskin isotiocyanate (FITC) is emitted in the green range, while the mattereritrin emits in the red range. The fact that there are different fluorores, capable of radiating at different wavelengths, allows a different Ag to be detected in the same cut of tissue or cell. Visualization of fluorescence can be performed with a fluorescent microscope (for tissue incisions or slides) or cytometry flow (for hanging cells). INDIRECT IMMUNOMARCATION Secondary Ac Slide Cells marked Ac Primary Ag 10. 9 Flow cytometry Flow cytometry (CMF) is a highly efficient procedure for characterizing cell populations that are in suspension. Initially, it was mainly used to determine the phenotype of cells, i.e. differential expression of antigens in the plasma membrane, but today there are many structural and functional parameters that can be measured by CMF (Figure 1). Among the many advantages it has regarding fluorescence microscopy are the ability to analyze a very large number of cells within seconds and the ability to quantify the intensity of fluorescence. The main drawback is the high cost of acquiring and maintaining the flow sensor. Figure 1: Flow cytometry has been used in biomedicine for various purposes: In hematology: cell contulation, white blood cell formula, cyticuloste contact, bone marrow analysis. In pharmacology: cellular kinetic studies. In immunology: subpopulation of lymphocytes, stimulation of lymphocytes. In oncology: diagnosis/forecast, treatment monitoring. In microbiology: bacterial and viral diagnosis, sensitivity to antibiotics. Flow cytometer: It is defined as a cytometer of flow to the machine, which is able to measure the components and properties of cells and cellular organelles (biological particles) flowing in the cell suspension. Cell sortulators have the same performance and capabilities as flow cytometers, but with the additional ability to selectively separate particles from liquid suspensions. Due to the specific characteristics of flow cytometers, the sample being analyzed should be in the form of a suspension There are samples such as peripheral blood, bone marrow, or other biological fluids that require minimal processing; solid tumors or paraffin samples, on the other hand, need more or less intense desegation (mechanical, enzimatic, etc.). For analysis, suspended cells are forced to pass, one by one, through a very thin thread. The laser beam affects each cell, which leads to the scattering of light in different directions. A series of photo-multhipel (FM) tubes detects diffuse light, providing information about cell size, detail or complexity, as well as fluorescence radiation in case the cell is attached to an antibody marked with fluorochrome. Below is a cytometer flow plan: 11. 10 Analysis and presentation of results: Cell size data, Cellular granularity and fluorescence (if antibodies marked with fluorochromes have been used) are analyzed using specific software and presented as graphs, of which are given the most common examples below: 1) point-of-size graph compared to granularity: examples of human peripheral blood white blood cells are shown in which regions corresponding to neutrophy (more complex) are clearly distinguished by granular lysos) monocytes (intermediate complexity, but larger size) and lymphocytes (small and non-pellets in cytoplasm). In this case, antibodies were not used to mark cells. Cell Size (FSC) Granulation (SSC) Peripheral white blood cells Neutrophils Ac lymphocytes with green fluorochromic current flow with cells, moving from FM to FM to FM-green fluorescence for FM red fluorescence for FM-size for granular cells marked ac fluorescent ac 11 2) Fluorescence histogram: An example of monocytatic styping cells is marked with a monoclonal antibody directed against the CD18 antigen shown. The antibody is marked fluorescein isocyanate (FITC) (green fluorescence) and is an isotype of IgG1 murin. As a non-specific communication control, cells are incubated, on the other hand, with IgG1 murine marked fitC, but not directed against any cellular antigen (isotype control). 3) Green fluorescence (FL1) compared to the red fluorescence (FL2) graph: An example of peripheral blood white blood from a patient with chronic B-cell lymphatic leukemia, which were simultaneously marked with 2 antibodies shown: FITC-labeled anti-CD4 and ficoeritron labeled anti-CD8 (PE) (red fluorescence, FL2). 3 populations differ: in the upper left quadrant are positive CD8 T cells, in the lower right quadrant, positive CD4 Ts and in the lower left quadrant cd8 or CD4 (B lymphocytes, NK cells). The absence of points in the upper right quadrant indicates the absence of cells that simultaneously express CD4 and CD8. If the thymus cells were marked, most of the dots would be in the upper right quadrant. CD18-FITC Green fluorescence (FL1) Number of unsigned cells (isotype control) CD18 positive CD4 and CD8 peripheral blood lymphocytes in a patient with CD4-FITC CD8-PE B-cell leukemia 13. 12 3.b. RADIOIMMUNOANLISIS (RIA) and RADIOIMMUNOMETRIC TECHNIQUES (PRIST, RAST) Radioinanoanalysis Method (RIA) allows to quantify the presence of a small amount of this Ag or Ac in a biological sample. Although it is a sensitive method of summing up, it is not currently as widely used as it used to be before, and has been replaced by other methods that do not use radiostops. The technique is based on the competitive inhibition of the union of radioactively labeled Ag with its specific ac, identical to the non-target Ags present in the sample for analysis. To perform the technique should be counted in advance with: 1) Specific Acs vs. molecule that will quantify (that molecule may be Ag or immunoglobulin), 2) molecules that will quantify the radiolbel and 3) sample to be analyzed (where the molecule that will be quantified is located). As can be seen from the diagram, immune complexes formed by the marked molecule (Hot Ag) and a specific antibody collide with a pattern that has an unmarked molecule (Cold Ag). If the concentration of cold Ag is higher, the displacement of the hot Ag will occur, and so the radioactivity of the Ag-Ac complexes will decrease, while the radioactivity of the free Ag faction will increase. By performing the standard curve with known free Ag concentrations, Ag concentration in the biological sample can be calculated. Radioimmunometric techniques allow you to detect Ag present in a sample using antibodies, conjugated to radioactive isotopes. They should not be considered synonyms of RIA, as they are not based on competitive braking by shifting hot Ag. The PRIST (Paper Disc Radio Immunosorbent Test) is used to measure igE levels in serums. IgE serum is usually present in low concentrations and increases, for example, in allergic patients. As shown in the picture, solid support (paper disk) is used in which Ac anti-IgE (antibody capture) is adsorbed. Paper discs are incubated by the patient's serum, and ac IgE, present in the serum, will be connected to a specific ac paper disk. After incubation and washing, an anti-IgE Ac is added, conjugated with a radioactive isotope that recognizes IgE attached to grip antibodies. After incubation and washing reading paper disk. The results are compared with the results obtained in the calibration curve in order to determine the concentration of IgE in the serum. RAST (Disk RadioAllergoSorbent Test) method is used to dose specific igE levels for a specific allergen. The allergen is absorbed on the paper disc and then the allergic patient's serum is incubated. If specific IgEs are present in the serum, they will be attached to the allergen and then recognized as Acs anti-IgE conjugated with radioactive isotopes. After incubation and washing, the paper disk radioactivity readings are performed and the results are compared with the results obtained in the calibration curve to determine the specific concentration of IgE in the serum. Ag: Hot antigen. Ac: specific antibodies. Ag: Cold antigen. Radioinmunoanálisis (RIA) Ag-Ac Ag-Ac Ag-Ac Ag-Ac Ag-Ac Ag-Ac Ag-Ac Ag-Ac Ag-Ac Ag-Ac Ag-Ac + Ag Ag Ag Ag Ag Ag Ag Ag Ag Ag Ag Ag Ag Ag Ag Ag Ag Ag Ag Ag 14. 13 3.c. ELISA (Enzime Linked ImmunoadSorbent Assay) The ELISA method uses Acs, marked with an enzyme (usually peroxidase) to visualize ag-Ac reaction. The technique is used to identify both Ags and Acs. The definition of Ag ELISA The most common METHOD of ELISA to identify Ags is the direct model of the ELISA Sandwich. In this model, the Ag-specific Ac of interest adsorbed into solid support (Petri plate), on which a biological sample will be added. In case Ag is in this sample, it will be caught on the plate and will be exposed after adding another particular Ac conjugated to the enzyme. Finally, a colorless substrate substrate is added, which, under the influence of the enzyme, will produce a color product that will produce a color observed by the naked eye and quantifiable by the spectrophotometer. Added specific IgE patient serum is present in the serum of the patient Added from the radioactive labeled Ac anti-IgE paper disc with the adsorbed allergen Reading radioactivity RAST Acs anti-IgE radioactive serum aggregate IgE patient, Present in the serum of the patient Added from the radioactive marking Ac anti-IgE Paper disc with Ac.de capture anti-IgE radioactivity reading PRIST Acs 14 Definition of Acs ELISA To identify specific Acs for this Ag , indirect modalities of ELISA, where Ag interest is adsorbed in the ELISA plate is commonly used. They can be used as antigens, viral or bacterial proteins and even complete viruses, but it is increasingly commonly found exclusively adSorber proteins of immune interest. This is one of the methods of choice for finding Acs against HIV virus proteins in a patient's serum. The steps of technology are laid out in the 4. OTHER IMMUNOLOGICAL TECHNICALS 4th ELECTROFORTIC PROTENYGRAM ElectroFORTIC Prote'nogram is a simple laboratory technique that allows you to divide into 5 fractions or groups of proteins present in a particular biological fluid (serum, urine, etc.). The technique is based on the electrophoretic separation of various proteins by applying an electric field to support agar soda or cellulose acetate. Once the electrophoretic run is completed, the protein strips can be displayed after using protein dyes. Subsequently, the bands observed by the naked eye (Figure A) can be quantified by a densitometer, which quantifies the intensity and width of each band (Figure B), and the percentages of each of the five protein fractions can be obtained. The various factions we can find are: Albumin, alpha 1 globulina (a1), alpha 2 globulins (a2), beta-globulins (ß) and gamma-globulina (γ). ALBUMIN: This is a protein that is synthesized in the liver and is the highest concentration in serum. 1: There are numerous proteins in this fraction, among which we can mention alpha 1-antitrypsin and alpha 1-anti-antiochometripsin 16. 15 No 2: This faction includes haptoglobin and C-reactive protein. The question β: in this faction we can find, among other things, fibronectin, transferrin and beta 2 microglobulin. γ: this fraction contains immunoglobulins, which can decrease in certain immunodeficiency and increase in certain chronic inflammatory processes (rheumatoid arthritis, systemic lupus erythematosus), infections and allergies. 4.b. NEFEDOMETRY. Immunoglobulins IgM, IgG and IgA can also be quantified by non-feulometry. To do this, incognito serum must be incubated with an excess of specific antibodies (anti-IgM, anti-IgG or anti-IgA) in order to form immune complexes. This method is an optical method that quantifies light dissipated formed by immune complexes that are proportional to the concentration of a particular antigen (in our case, IgM, IgG or IgA unknown serum). 4.c. WESTERN BLOT is a very sensitive method that allows the identification of Ags and Acs. As seen in the figure, the technique of Western Blot is based on the electrophoret division of proteins into polyacrylamide gels, transfer (blotting) of these proteins to solid support (nitrocellulosic membrane or nylon) and subsequent detection of one or more specific bands defined by Acs. Protein migration is carried out in the presence of detergents (Dodekial sulfate sodium, SDS), so their migration depends primarily on the molecular weight (PM) peptides. Thus, the described method allows to investigate the presence of this antigen in the mixture of proteins present in this sample, and for this it must be organized Ac specific to mark the membrane. However, the Western Spot method is also very useful for detecting specific acs in serums. If specific Acs are to be detected, Ag Interest should be counted separated by electrophoresis and transferred to a membrane that will be incubated with serum for analysis. This membrane must be incubated with a second antibody marked with an enzyme in order to detect specific bands. Western Blot technique to determine the specifics of the HIV acs vs virus. Ag is a confirmation test of choice for cases where serum patients have tested positive for ELISA 17. 16 5. HISTOCOMPATIBILITY ANTIGEN TIPIFICATION TECHNIQUES Goals: 1. Research on histocompatibility for vascular organ transplantation 2. Histocompestability studies for bone marrow transplantation 3. Paternity research 4. HLA allele association of studies with diseases (susceptibility) 5. Anthropological studies to establish possible relationships between different populations or ethnic groups. Classification of input methods 5th place. Serological methods: microlimphocytotoxicity in Terasaki 5.b. Molecular methods a. PCR with certain sequence feeders (SSPs) b. PCR with locus-specific lures and hybridization with marked, Allele-specific probes (SSOP) c. Direct Sequencing (SBT) d. Others: RFLP, SSCP Proteins with SDS Polyacrylamide Gel Direction of Migration Proteins after being separated by PM in the polyacrilamine membrane membrane membrane Gel Membrane subsequently marked specific Acs Membrane with transferred proteins Added from the first Ac Wash and the addition of the ac 17 5a. SEROLOGICAL TECHNICALS: Microlyinfocytotoxicity in the plates of the Telasaki Foundation: This method is based on the use of specific antibodies against various HLA alleles (molecules I and II grades). When these antibodies recognize the antigen against which they are specific, and when there are additives there is cellular lys. The test is performed on a 60-pit plastic plate called Terasaki plates and cell death quantitatively differential staining of viable and dead cells. Stages: 1. Patient blood test 2. Mononuderal cell insulation (Ficoll centrifugation®) 3. The planting of cells in the body-body plate pits was previously sensitized with polyclonal serums against various HLA alleles. 4. Incubation (binding A to Class I or Class II HLA molecules) 5. Added additional font (Rabbit Serum) 6. Incubation and lys cells that joined Ac at the previous stage 7. Adding a contrast dye to differentiate living and dead cells (fluorescein diacetate) 8. Microscopic observation and classification. Scheme Technique Variants: Anti immunoglobulin 1. Patient's blood draws 2. Mononuderal cell insulation (Ficoll centrifugation®) 3. Sowing chambers in the pits of the Plate Telasaki No. 4. Incubation (ac binding to class I HLA molecules) 5. Incubation with human anti-immunoglobulin rabbit IgG 6. Add-on font 7. Incubation and lys cells that have joined Ac 8. Adding a contrast dye to differentiate living and dead cells (fluorescein diacetate) 9. Microscopic observation and classification or scoring Considerations: The Origin of Serum: 1. Serum women multipara (4 or more children of the same parent), since during childbirth the passage of blood comes from the fetus to the mother, and that allows the semigenic cells of the fetus to act as 19. 18 immunogens in the mother, which will mount an immune response against the father's HLA alleles that fetal cells express. 2. Serum patients who have rejected a previous transplant where a humoral (and cellular) immune response directed against the alleles of HLA expressed during transplantation are usually observed. 3. Serums of polytransform patients, as in the blood transfusion there are donor white blood cells, which will act as immunogens in the over-filled patient. Pros: - Simple technique requiring a small complex equipment (inverted epifluorescent microscope) Disadvantages: - The technique should be performed without interruption from start to finish, as it works with viable cells. No optional ac supplement retainers are detected, except for the option using anti-immunoglobulin - High Cost - Low Resolution - No serums vs. rare alleles - Most serums are not monospecific, and also have cross reactivity with other alleles. High homoology and identity between many epitopes of Class I and Class II HLA molecules allows you to group these molecules into cross-reactivity groups or CREGs - not suitable for input from cadastral donors (especially in the case of bone marrow transplantation) - Difficult interpretation due to a lack of monograms and cross-reactivity - Gomozigoz is not detected (in general 1 allele reported and the other is reported as white) - Ambiguity (no homozigosis is detected (a total of 1 allele reported and the other is reported as white) to identify certain combinations of alleles) 5.b. MOLECULAR TECHNICALS: SSP Foundation: In this method of polymerase, the chain reaction (PCR) is performed using as a form of DNA extracted from the patient's peripheral blood cells. Specific olygonnucleotic combinations of olygonnucleotides are used for various HLA alleles as a primer for olygonnucleotics. In this way, DNA fragments of different lengths are strengthened. The resulting DNA fragment, which is analyzed in the electrophoresis of the agar gel, allows allele to the sample analyzed. This means that a significant number of PCR reactions must be performed for each sample (the same number of first pairs are used, which in turn will be reflected in the degree of resolution reached). Stages: 1. Patient blood test 2. DNA isolation 3. Chain reaction polymerase (PCR) with different combinations of the first 4. Electrophoresis in agarosa gels containing ethyde bromide 5. Monitoring the structure of reinforced DNA bands in PCR under ultraviolet light 6. Integrating the results 7. The purpose of alleles to the sample analyzed the General Plan of Technology 20. 19 Considerations: Benefits: - Simple technique that requires a little sophisticated equipment (PCR-machine) - does not require cell insulation (working with whole blood) - Resolution more, than serological methods (Intermediate resolution, which means that some combinations of alleles can not be solved) - Relatively low cost - the technique can be discontinued at various stages (after DNA extraction) , after PCR or after electrophoresis) - Allows to detect zero alleles Disadvantages: - Not suitable for input of cadavers (especially in the case of bone marrow transplantation) , because the achieved resolution is intermediate - Ambiguity (certain combinations of alleles can not be defined) - Each pair of first allows to amplify one or more alleles of HLA, so due to the high polymorphism of this system, it will be necessary to use a large number of pairs first to obtain an acceptable resolution when distributing alleles in the sample. Therefore, each sample requires a large number of PCR reactions (which are performed simultaneously). Thus, the method is not practical for the simultaneous analysis of a large number of samples. No gomozigoza was found. SSOP Background: In this method, chain reaction polymerase (PCR) is carried out using DNA extracted from the patient's peripheral blood cells as a form. Specific olygonucleotides are used as a primer of olygonnucleotides for

various HLA loci (HLA-A locus, HLA-B locus, HLA-C locus, HLA-C locus, etc.) are amplified separately. Enhanced DNA fragments are then immobilized on nylon membranes and hybridized with marked probes (olygonnucleotics) specific to various HLA alleles. A diagram of signals received according to the probes that reacted is detected using radiographic plates, allowing the allele to be assigned to a sample analyzed. Stages: 1. Patient blood test 21. 20 2. DNA isolation 3. A chain reaction polymerase (PCR) with different specific firsts for different HLA 4 loci. Electrophoresis in agarosis gels to check the response quality of PCR 5. PCR products in nylon 6 membranes. Hybridization of membranes with allele-specific probes marked 32P 7. Exposure to membranes on X-ray plates 8. Analysis of the structure of signals received in membranes 9. Integration of the results 10. Assigning alleles in the sample analyzed General Methods of Sketch Considerations: Benefits: - Does not require cellular isolation (working with all blood) - High resolution - Technique can be discontinued in various steps - Using quite a large number of probes, homosigosis can be detected , detection of hemoluminescence can be performed, so that no radioactive waste is generated - Allows detection of zero alleles - allows simultaneous analysis of a large number of samples Flaws: - Complex interpretation (cross reactivity between probes , the detection of certain combinations of alleles produce similar models in autoadiographic plates) - The conditions of washing different probes can be different, which complicates in the simultaneous processing of many samples - Despite their high resolution, ambiguities remain (although they are much less than in the above methods) - Complex and expensive equipment that requires somewhat more complex equipment (PCR machine, hybridization of furnace, radiooscope processing infrastructure) - SBT Foundation radioactive waste generation: 22. 21 In this method, chain reaction polymerase (PCR) is carried out using DNA extracted from the patient's peripheral blood cells as a form. Specific olygonucleotides are used as a primer of olygonnucleotides for various HLA loci (HLA-A locus, HLA-B locus, HLA-C locus, HLA-C locus, etc.) are amplified separately. Enhanced DNA fragments are then sequenced directly using an automatic DNA sequencer, which also analyzes the resulting sequences and, compared to the internal database, assigns the HLA alleles to the analyzed sample. Stages: 1. Patient blood test 2. DNA isolation 3. A chain reaction polymerase (PCR) with different specific firsts for different HLA 4 loci. Electrophoresis in agarosis gels to check the response quality of PCR 5. Perform a sequence reaction using the first, marked with orochroms, suitable for the type of detector possessed by sequencer 6. The sequence analysis received 7. Comparison with the sequence database used by the device and the integration of the results 8. Assigning alleles to a sample analyzed by General Sketches of Techniques Considerations: Benefits: Cell Isolation (working with whole blood) - High resolution (maximum possible resolution achieved) - Technique can be discontinued in various steps - Ideal for detection of gomosigosis - Optimal for cadaver donor typing - Automated interpretation - Allows you to detect zero alleles Flaws: - Complex technique requiring complex and expensive equipment (automatic sequence) 22 6. CROSS-MATCH. Purpose: Determine the presence of specific serum HLA allel antibodies of patients on the waiting list for vascularized solid organ transplantation. Modalities: 6th place. Final cross-match vs. donor: The goal is to analyze the presence of serum antibodies specifically directed against the potential donor's HLA alleles. Analyzing the presence or absence of these antibodies is crucial to deciding whether or not a transplant is performed. This is because the presence of this type of pre-formed antibodies in the recipient of the transplant inevitably leads to hyperagit rejection. This method can be performed micro lymphocytoxicity in Terasaki plates or cytometry flow. In the first case, the recipient's serum collides with peripheral blood cells from a potential donor (if it is a living donor) or with spleen cells or lymph nodes (if you are a cadapher donor). After incubation, the source of the supplement is added and the technique described in Microinfocytotoxicity in Telasaki plates continues. In the case of the final cross-match against the flow of cytometry give, monoyander peripheral blood or spleen or lymph nodes of the cells of a potential donor, with the serum receptor collide. After incubation, the system is revealed with human anti-immunoglobulin goat or rabbit immunoglobulins marked with fluorochrome (phtorshane isotiocyanate commonly used). Finally, the cells are analyzed in the cytometer of the stream. 6.b. Cross-match vs. panel: Aims to analyze the presence of serum antibodies against various HLA alleles from a potential donor. This method is usually performed microlinfocytotoxicity in Terasaki plates, where the serum receptor collides with peripheral blood cells of various individuals whose HLA alleles are representative of the population (usually using 20 heterozygous individuals in order to cover 40 alleles of each locus). After incubation, the source of the supplement is added and the technique described in Microinfocytotoxicity in Telasaki plates continues. This method calculates the percentage of cells that were poured in the test, which is equivalent to the number of alleles against specific Ac, expressed as a percentage of the total number of alleles analyzed during the trial. The calculated setting is called PRA (reactive antibody panel), and it has been noted that there is a link between PRA and the likelihood of success or failure of a kidney transplant. 7. IMMUNOLOGICAL TECHNIQUES TO STUDY THE FUNCTIONALITY OF FAGOCYTES 7th Nitroblue Tetrazolium Reduction Trial (NBT) This study evaluates the ability of microbicides of certain immune system cells by converting a colorless chemical compound, NBT, into an intensely colored compound. For this test, blood is taken from the patient and the procedure is performed to separate polymorphono cell cells (mostly neutrophils). NbT is added to these cells. Neutrothylos have the enzyme NADPH Oxidase, which is capable of generating reactive oxygen mediators that attack microorganisms. These reactive oxygen mediators are also responsible for turning the colorless NBT into a dark blue compound called Formazan. This is displayed by looking at a patient's neutrophils under a microscope to see if they have a dark blue compound. If the patient's neutrophils have any changes in the functioning of the nadPH oxidase enzyme (as in those with chronic granulomatous diseases), they can ingest bacteria but not destroy them and therefore will not be able to cause a change in the color of the NBT. In a healthy person, more than 95% of neutrophils are able to reduce NBT. In most patients with chronic granulomatous diseases, only 20% to 80% of neutrophils are able to reduce NBT. In cases where clinical suspicions of chronic granulomatous disease are high, more specific tests should be carried out on oxidative metabolism of neutrophil neutrophil (oxidation dyes producing fluorescent compounds, etc.). The use of the drug D-peninciamine can give abnormal results in the trial of reducing NBT, as if it were a chronic granulomatous disease. 24. 23 7.b. Microbicide Analysis This study assesses the phagocytic function of immune system cells. It requires that these cells, to be able to phagocytes and make a breathing explosion, depends on the enzyme nadPH oxidase. For this test, blood is taken from the patient and the procedure is performed to separate polymorphonogluary (mostly neutrophil) or monocyte cells. Opsonized bacteria (Staphylococcus aureus) are added to these cells, incubated at 37oC and samples are extracted at 30-minute intervals within 2 hours. These plaque samples on the blood agar and are incubated throughout the night at 37oC. This allows bacteria to grow and form visible colonies. Thus, the number of colonies for each mining time is calculated and number of colonies against time. It is to be expected that as the incubation time is longer, the number of colonies is lower because the patient's cells have phagocyte bacteria and therefore fewer and fewer bacteria. Agar is a nutritious cultural environment (looks like gelatin). In particular, the agar of blood is enriched with blood and serves to detect some bacteria, such as Staphylococcus aureus, which are released by exotoxins called hemolysins, which can smooth out red blood cells. % Bacteria die time tipos de reacciones de precipitacion inmunologia