Ministry of Health of Ukraine
Poltava State Medical University

Approved"
at the meeting of the Department of
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Minutes № from
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METHODOLOGICAL RECOMMENDATIONS FOR CONDUCTING AND PREPARING FOR PRACTICAL CLASSES

Academic discipline	Clinical immunology and allergology
*Module **4***	Clinical immunology and allergology
Content module	Clinical immunology and allergology
Topic №7	Basics of transplantation and regeneration immunity
Course	5
Hours	2

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Methodological recommendations for the practical training for independent work of students in preparation for the practical training and during the class were prepared by:
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Methodological recommendations were re-approved at the meeting of the Department of Internal Medicine of Internal Medicine №3 with Phthisiology_____
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1. Relevance of the topic.

At the turn of the 21st century, medical science has made significant progress: medical technologies are becoming more effective; problems that seemed unsolvable yesterday are being successfully overcome today; the prospects for the application of certain methods are expanding. Without a doubt, such a field of medicine as transplantology is becoming a symbol of the new century.

The idea of organ transplantation was dictated by the prospect of replacing a diseased organ with a healthy one. For the first time in the world, an organ (kidney) transplant was performed by Professor Y. Voron in 1935 in Kharkiv (more precisely, he transplanted a donor kidney into the femoral vessels of a woman poisoned by sulema). In 2004, the European Association of Urologists issued a new Guideline on kidney transplantation. It contains detailed recommendations on the main surgical and immunological aspects of kidney transplantation, defines surgical tactics and features under various circumstances and in the event of a number of complications, great attention is paid to issues of immunological compatibility, donor organ collection, immunosuppressive therapy, etc. The basis of successful immunosuppressive therapy is the principle of "survival balance", that is, the doctor must prescribe the dosage of drugs necessary to suppress rejection, and at the same time not increase the overall risk to the life and health of the recipient.

General goal: To study the technique of clinical and immunological selection of a donor-recipient pair. Identify the type of allograft rejection crisis for proper management of the recipient after transplantation.

Specific goals:

1. Determine the immune status of the recipient and the donor.
2. To assess the degree of histocompatibility (HLA - phenotype) of the recipient and the donor.
3. Prescribe adequate immunosuppressive therapy after transplantation.
4. Identify infectious complications in the recipient after inadequate immunosuppressive therapy.

Theoretical questions for practical training:

1. The main types of transplants.
2. Determination of the degree of histocompatibility in the donor and recipient.

3. Determination of different temperature pre-existing antibodies in the recipient to antigens of the donor's HLA system (anti-T and anti-B antibodies).
4. Determination of anti-endothelial antibodies in the recipient to antigens of the donor.
5. Types and immunopathogenesis of rejection crises.
6. Immunological monitoring of the recipient after transplantation.
7. Immunosuppressive therapy after transplantation.
8. Infectious complications in allograft recipients.

Approximate basis of action

There are the following types of transplantation:

1) autotransplantation — transplantation of one's own tissues;

2) allotransplantation — transplantation of organs and tissues within the limits of the same biological species;

3) xenotransplantation — transplantation of organs and tissues within different biological species;

4) isotransplantation - transplantation between identical twins or between genetically identical animals. The subject to whom the transplant is transplanted is the recipient, and the one from whom the organ or tissue is taken is the donor.

For the first time in Ukraine, regular allogeneic kidney transplants began in 1973 at the Institute of Urology and Nephrology of the Academy of Medical Sciences of Ukraine under the leadership of prof**. V.G. Karpenko. Later, this work was headed by prof. E. Ya. Ram.**

SELECTION OF THE DONOR - RECIPIENT PAIR

Due to the fact that the donor's cells carry antigens on their surface that differ from the recipient's antigens, the latter's immune system develops an immune response to the transplant. As a result, a graft rejection reaction is formed.

A method that reduces the rejection reaction to a greater or lesser extent is the selection (selection) of the donor-recipient pair based on histocompatibility antigens, which in humans are combined in the HLA system (Human leukocyte antigens). They are also called transplant antigens

In the practice of transplantation immunology in Ukraine, typing, i.e. determination of the HLA phenotype of the donor and recipient, is most often carried out by antigens of loci A, B, C, DR. Selection involves the selection of the most compatible donor and recipient.

A histocompatibility index was proposed to assess the degree of histocompatibility. With one identical HLA system antigen in the recipient and the donor, the histocompatibility index is 25%, with two - 50%, with three - 75%, with four - 100%. At the same time, the degree of histocompatibility according to the antigens of the so-called classical HLA loci is evaluated.

Some antigens of the HLA system are similar in structure (the sequence of amino acid residues has a certain degree of homology). The presence of such similar antigens in the donor can increase the degree of histocompatibility.

There are several groups similar in structure to HLA-antigens, which are called cross-reactive:

On locus A — A1, 3, 11; A2, 28; A23, 24; A25, 26; A30, 31;

On locus B—B5,35;B7,22,27;B8,14;B13,40;B15,17;B38,39;B12,21.

Based on these HLA features, it is possible to improve the results of donor graft selection by HLA. It was established that the presence of antigens of the HLA system with strong cross-reactions in the donor increases the histocompatibility index by 20%, with less strong ones by 10%.

Selection actually pursues the selection of such a donor-recipient pair, in which the donor differs from the recipient in the least way in terms of antigens of the HLA system.

In order to identify the HLA-phenotype, the peripheral blood lymphocytes of the donor and the recipient are typed.

To type lymphocytes according to class I antigens (HLA-A, B, C), a lymphocytotoxic test in the micromodification of Paul Terasaka is used. The ingredients are anti-HLA active sera that make up the typing panel, peripheral blood lymphocytes of the subject, normal rabbit serum (as a source of complement).

To detect class II antigens (HLA — DR, DP, DQ), a prolonged lymphocytotoxic test is used with a suspension of cells enriched with B-lymphocytes, on the surface of which these antigens are presented. As you know, the peripheral blood of a person contains only 5-20% of B-cells, which is not enough to perform the test. There is a method of obtaining a suspension of lymphocytes enriched with B-cells, which is based on the property of B-lymphocytes to attach to the fibers of synthetic cotton wool.

Preexisting antibodies. According to the well-known rule, the transplantation of an allogeneic organ is strictly prohibited if the recipient has pre-existing antibodies to antigens of the donor's HLA system. Preexisting antibodies are produced as a result of sensitization of the recipient by antigens of peripheral blood lymphocytes. In general, preexisting antibodies can be detected in about a third of the human population as a result of blood transfusions or pregnancy. By their action, they are mainly lymphocytotoxic antibodies.

Pre-existing antibodies specific to the lymphocytes of a particular donor are detected in a conventional lymphocytotoxic test (donor lymphocytes and recipient serum). The reaction in this execution was called cross-match, or cross-match. Preexisting antibodies are a risk factor for subacute (and to some extent, acute) graft rejection and are considered a negative prognostic indicator.

The activity of pre-existing antibodies in a cross sample is indicated by the cytotoxic index, %. Traditionally, a cytotoxic index > 5% is taken into account, which means 5 dead lymphocytes per 100 lymphocytes in the field of view.

Detection of non-specific cytotoxicity (that is, cytotoxicity not to the donor's lymphocytes, but to a set of lymphocytes from different individuals) is not a direct contraindication to transplantation, however, it is considered as a negative prognostic sign and requires careful monitoring of the recipient after transplantation. A high percentage of positive samples (more than 25%) indicates a massive sensitization of the potential recipient. In this case, well-known rules follow: a recipient with a wide range of preexisting antibodies needs to find a donor to whose lymphocytes this recipient would not have specific preexisting antibodies. At the same time, the degree of HLA histocompatibility between the donor and the recipient can be disregarded. This should be understood as the incompatibility of the donor-recipient pair in this case is less dangerous than the risk of an acute rejection crisis due to a high level of preexisting antibodies.

MECHANISMS OF ALLOTRANPLANT REJECTION

As indicated above, an allograft endowed with foreign antigenic structures initiates an immune response in the recipient. As a result of this, a rejection reaction develops, which in the clinic is called a rejection crisis.

According to the clinical picture of the rejection crisis, its classification was proposed, which corresponds to certain immunological features.

Rejections are distinguished:

1) superacute, which develops immediately after connecting the transplant to the recipient's bloodstream;

2) acute, developing during the first three weeks after transplantation;

3) chronic, observed after several months or years.

The mechanism of hyperacute rejection is caused by presensitization of the recipient to antigens of the donor's HLA system, that is, it is associated with the presence of preexisting antibodies in the recipient. As already noted, the material substrate of presensitization is antigens of the HLA system, which induce a humoral immune response in the recipient even before transplantation due to hemotransfusions, pregnancy or treatment with programmed hemodialysis. The function of the allogeneic kidney (excretion of urine) in the case of an immediate rejection crisis stops in the first minutes or hours after transplantation. Evidence that pre-existing antibodies are the cause of a hyperacute rejection crisis is the fact that they disappear from the recipient's peripheral circulation immediately after connecting the graft to the bloodstream. These antibodies are fixed in the transplanted kidney, as evidenced by the results of immunofluorescence and electron microscopy.In a hyperacute rejection crisis, pre-existing antibodies damage the transplant either as a result of their direct impact, primarily on the endothelium of the renal glomerulus capillaries by the mechanism of complement-dependent lysis, or in connection with the development of immune inflammation with the participation of the complement system, which is accompanied by hemocoagulation disorders. At the same time, the antigen-antibody complex formed when antibodies fixate on the antigenic determinants of the cells of the allogeneic kidney activates the complement, resulting in an aggressive effect on the cells of the capillaries of the kidney glomeruli. Immune inflammation develops, which includes the mechanisms of hemocoagulation, which leads to the deposition of fibrin and the formation of blood clots in the vessels of the transplant. Violation of blood flow in the transplant leads to rejection — the final result of an immune conflict.

Mechanism of acute rejection.

In the pathogenesis of an acute crisis of rejection, which occurs as a result of insufficient immunosuppressive therapy, the main role belongs to the cellular chain of immunity, although the participation of humoral reactions cannot be categorically denied. The pathogenesis of an acute crisis of rejection is based on the principle of immunological recognition of "alien". The initial stage of this mechanism is the stage of recognition of a foreign antigen, and the final stage is the interaction of the effector cell, in this case, the CD8+ T-cell (sensitized cytotoxic lymphocyte), with the target cell and the implementation of the killing effect.

Immunological recognition of the antigen of the donor's HLA system occurs in two ways: direct and indirect.

Direct recognition is based on the fact that antigens of the donor in the form of peptides are presented for recognition to T-lymphocytes of the recipient by antigen-presenting cells of the donor itself. As a rule, they are the so-called "passenger leukocytes" with the phenotype of dendritic cells. These are leukocytes that remained in the donor kidney after its removal from the donor's body. The latter, as is known, carry HLA molecules of class I and II. Thus, conditions are created for stimulation of both T-helpers (CD4+ cells) and T-killers (CD8+ cells) of the recipient's lymphocytes. Recognition of peptides presented by molecules of HLA II class of antigen-presenting cells of the donor is carried out by the antigen-recognizing receptor of the T-helper of the recipient. Recognition of donor antigens presented by HLA class I molecules expressed on passenger lymphocytes is carried out by the antigen-recognizing receptor of T-killers (DM8+ cells) of the recipient.Stimulation of these recipient lymphocytes initiates the maturation of specific T cells, i.e., a cellular immune response with the realization of an acute rejection crisis in the 1st week after transplantation. Stimulation of the recipient's helper T-lymphocytes promotes the development of both cellular and humoral immune responses. Thus, the direct recognition of donor antigens is based on the fact that antigen-recognizing T-lymphocytes of the recipient are "offered" ready-made antigenic determinants of the donor by donor antigen-presenting cells.

Indirect recognition of donor antigens is based on a general biological principle: processing of donor antigens and presentation of peptides are carried out by antigen-presenting cells of the recipient. In this case, both a cellular and a humoral response to the donor's antigens is also initiated, but the immune response develops more

slowly and an acute rejection crisis is observed on the 2nd or 3rd week after transplantation.

Extremely important is the fact that depending on the type of stimulated T-helpers, either a cellular or a humoral immune response is initiated. As already mentioned, there are two types of helpers. The first (type 1 T-helpers) help the precursors of killer T-lymphocytes to differentiate into sensitized T-lymphocytes, and the second (type 2 T-helpers) help B-cells to differentiate into plasma cells. Data from morphological studies indicate that the acute crisis of rejection is the result of stimulation, mainly of type 1 T-helpers, since rejection is accompanied by a cellular immune reaction.Thus, in kidneys removed as a result of an acute rejection crisis, there are cellular infiltrates that are initially of a focal nature and are provided by mononuclear cells (lymphocytes, cells of the plasmacytic series). Then comes total infiltration mainly by mature lymphocytes. Further infiltration by macrophages and segmented nuclear cells indicates the completion of the rejection process, the end of the immune conflict.

Mechanism of chronic rejection. The development of chronic transplant rejection is possible several months or even years after the transplantation of an allogeneic organ, most often due to insufficient immunosuppressive therapy. Humoral antibodies to antigens of the donor's HLA system are mainly involved in the pathogenesis of chronic transplant rejection. There is a correlation between the level of humoral antibodies and the development of vascular changes with their obliteration. In contrast to the acute crisis of rejection, which is characterized by intense cellular infiltration of the transplant, in chronic rejection it is weakly expressed, and plasma cells prevail in the infiltrate and fibrosis of the interstitial tissue is observed. From ultra-acute chronic graft rejection, it differs in the absence of fibrin thrombi in the vessels, despite the presence of antibodies. This is explained by the fact that the concentration of antibodies is insufficient for the development of an acute immune inflammatory reaction with the formation of massive fibrin thrombi, as in hyperacute rejection. However, an increase in urinary excretion of fibrin/fibrinogen breakdown products indicates that fibrin is still formed, but is immediately lysed. Therefore, chronic graft rejection is characterized by gradual damage and obliteration of the lumen of vessels — arteries and arterioles, as well as glomerular and tubular capillaries. The slow course of chronic transplant rejection and gradual vascular damage is accompanied by the deterioration of the function of the transplant and the replacement of the kidney parenchyma by fibrous tissue.

IMMUNOSUPPRESSIVE THERAPY IN ALLOTRANSPLANTATION

The modern scheme of prevention and treatment of rejection crisis most often includes: 1) azathioprine (Imuran) — an antimetabolite of protein synthesis; 2) corticosteroids — prednisone, dexamethasone, hydrocortisone, etc.; 3) cyclosporin A (sandimun).

Azathioprine (Imuran) mainly suppresses the cellular response by suppressing the induction of immune killer T lymphocytes. It is prescribed in a dose of 2-3 mg/kg of body weight per day.

Corticosteroids affect the recipient's immune system, suppressing macrophages, T-lymphocytes, synthesis of complement and cytokines. This allows you to stop the acute crisis of rejection at the beginning of its development. The most widely used is prednisone. To prevent an acute crisis of rejection immediately after transplantation, 3-4 mg/kg of body weight per day are prescribed until the recipient's clinical condition stabilizes; the maintenance dose is 0.5 mg/kg. In order to treat an acute rejection crisis, steroids are prescribed in very high doses: methylprednisolone - up to 1000 mg per day for 1-4 days. One of the mechanisms of the immunosuppressive effect of glucocorticoids can be the inhibition of IL-1 and IL-2 production, as well as a decrease in the expression of products of the main histocompatibility complex on the membrane of lymphocytes (for more details on the mechanisms of action of glucocorticoids, see the corresponding chapter).

Cyclosporin A opened a new era in transplantation, which was called the "cyclosporin era". It began in 1982. According to the Institute of Transplantology and Artificial Organs of the Ministry of Health of Russia, two-year survival of an allogeneic kidney with the use of cyclosporine was observed in 71% of cases compared to 49% without it.This result is explained by the fact that cyclosporine affects T-helpers directly, inhibiting their production of IL-2 and, thus, prevents the maturation of specific T-killers (DM8+ cells). This mechanism of action of the drug determines the success of transplantology during the "era of cyclosporine".

The average daily dose of cyclosporine is 5 mg/kg of body weight. Given the high nephro- and hepatotoxicity of the drug, it is necessary to monitor its content in the recipient's peripheral blood, which provides a radioimmunoassay method using Cyclosporin RIA-kit sets. The dose of cyclosporine in the vascular bed should be in the range of 200-400 ng/ml, taking into account the individual condition of the patient in the dynamics. The use of cyclosporin should be combined with the use of other immunosuppressive drugs (corticosteroids, cytostatics, etc.).

For immunosuppressive therapy, anti-lymphocyte serum (ALS), especially its globulins — ALG, was widely used due to the pronounced immunosuppressive effect. Currently, transplant centers in the West use ATG — anti-thymocyte globulin. Positive results in the treatment of acute rejection crisis are due to the fact that anti-thymocyte antibodies have a complement-dependent cytolytic effect on the T-lymphocytes of the recipient. In most well-known transplant centers, the use of ALS and ATS was limited due to the fact that due to the retention of protein material, the drugs caused severe allergic reactions. In addition, the experiment established that antilymphocyte serums act on the thymus, the central organ of immunity, causing its destruction. This requires great caution when using ALS and ATS in transplantology.

Recently, the drug OKT-3 was obtained, which is a monoclonal antibody against the SDZ component of the antigen-recognizing receptor of T-lymphocytes. Due to the blocking of the receptor, the T-lymphocytes of the recipient do not recognize the antigens of the donor and the immune response does not develop. Another mechanism of the immunosuppressive effect of OKT-3 is possible, which is realized due to the complement-dependent cytopathogenic effect of specific antibodies on T-lymphocytes.

All drugs used for immunosuppressive therapy have their drawbacks. The toxicity of cyclosporine has already been mentioned above.It is necessary to remember that immunosuppressive therapy suppresses protective immune reactions, deepening the state of secondary immunodeficiency, which most often occurs in the patient even before transplantation, which ultimately leads to infectious complications.

It is possible to assess the adequacy (adequacy) of immunosuppressive therapy using the dynamics of some immunological techniques. The most informative methods are:

1) determination of the number of T-helpers and T-killers/suppressors;

2) the quantitative ratio of these cells (immunoregulatory index), which should be within 1.0-1.3.

Test control tasks.

1. Patient M., 49 years old, underwent kidney allotransplantation. The histocompatibility index is 75%. After the kidney transplant, the patient was prescribed immunosuppressive therapy. On the 16th day after the operation, a fistula with a greenish discharge opened and signs of acute kidney failure suddenly appeared. In the immunogram: a significant decrease in the total number of CD3 and CD4 cells with an increase in the number of CD8 and CD19 lymphocytes, IRI-0.8. What threatens the patient?

a) infectious complication

b) an acute crisis of rejection

c) super acute crisis of rejection

d) chronic crisis of rejection

e) drug-induced glomerulonephritis

Correct answer: a)

2. Patient N., 47 years old, underwent liver allotransplantation. The histocompatibility index is 65%. On the 4th day of the postoperative period, pain appeared in the right hypochondrium, nausea, an increase in t to 38.10C. Immunogram: an increase in the number of CD3 and CD4 cells against the background of a decrease in CD8 lymphocytes, IRI - 2.2. In the punctate aspirate: T-helpers prevail, a high level of IL-2. What threatens the patient?

a) acute crisis of rejection

b) super acute crisis of rejection

c) chronic crisis of rejection

d) infectious complication

e) CMV-hepatitis

Correct answer: a)

3.Patient K, 47 years old, underwent allotransplantation of the liver due to total echinococcosis of the parenchyma. HLA phenotype of the recipient: A3, 7; B6, 15; C8, 12; DR 8, 10

HLA phenotype of the donor: A1, 28, B15, 21; C6, 8; DR 5, 7

Antigens A1 and A3 are cross-reactive

On the 4th day of the postoperative period, the patient felt the onset of pain in the right hypochondrium, nausea, bitterness in the oral cavity, as well as chills alternating with a feeling of heat, an increase in body temperature up to 38.10 C, headache, general weakness.

Blood biochemistry: AST 1.06 mmol/l, AlT 1.18 mmol/l, thymol test 14 units, alkaline phosphatase level three times higher than normal.

Immunogram: L – 4.2x109 /l, E – 1%, P – 3%, C – 63%, M – 7%, L – 26%, CD3+ 60.4 (N 62.8-85.0), CD4+ 61.3 (N 31.4-56.7), IPI – 2.2, CD19+ 19.2 (N 7.1-13.3), IgG – 17.3 g/l, IgA 1.21 g /l, IgM - 0.88 g/l, FI - 53%, FH - 6.5, CIC - 0.23.

Lymphoid cells predominate in the aspiration biopsy.

What correction should be applied?

1. antibacterial drugs

2. immunosuppressive therapy

3. enzyme preparations

4. antipyretics

Correct answer: b)