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THE LYMPHOCYTE BLAST TRANSFORMATION (BT) TEST

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ABSTRACT

The purpose is to show the rate of conversion of lymphocyte blast in different samples of human blood infected with myasthenia gravis (an autoimmune disease). This reaction was based on the ability of normal lymphocytes transformed in undifferentiated germ cells of blast type after culturing in vitro in the presence of specific allergens and nonspecific stimulants, mitogens (PHA or PHA, concanavalinA, lipopolysaccharides, and other substances). To determine the rate of conversion of the blast, the number of explosions and intermediate forms determined on 100 cells and a similar calculation is made in the control as well. The morphology of the human blood cells (lymphocytes and blasts) in the presence and absence of mitogen (phytohemagglutinin), which viewed under a microscope. As we all know, it was previously shown that under normal conditions of 40 to 90 percent of the lymphocyte can turn into explosions and reduce the conversion of the explosion in the presence of allergens, or PHA indicate any abnormality. Necessity biosafety diagnostic laboratory for analysis in each step of the test tube was fully considered in order to prevent / avoid the risk of any form especially for diagnostics of human blood at various ill patients. Therefore, in this document, different blood samples and serum were analyzed separately; Templates colony, the number of blood cells and their morphology were studied by light microscopy, to lead us to more understanding of the mechanism underlying immunopathology / immunodeficiency and their risks in the human immune system. Biosecurity measures to avoid infection and to avoid invalid results were fully met.

KEYWORDS: Biotechnology, Recombinant DNA technology, Transformation of lymphocytes blast mitogen.

INTRODUCTION

The twentieth century was marked by intensive development of functional science and particularly in the development of molecular biology and biotechnology.

Latest technologies allow the biological reconstruction of genetic systems, create new genetic constructs and widely used microorganisms in practice. Creating transgenic organisms allowed for progress in getting new resistance to various diseases of plants and animals. The development of biotechnology has led not only to the appearance of previously existing process, but also to change our outlook, medical practice and housekeeping.

However, the latest advanced technologies involve new biological risks and threats. This in turn triggered protests among the population with further development of biotechnology.

A new challenge to our civilization is associated with the understanding and analysis of the prospects for further development on one hand and the risks of biotechnology and biological hazards potentially in new technologies. In this connection is the analysis of the potential risks of working with the microorganisms, the DNA technology and the development of medical practice, including studies of autoimmune pathologies.[54,55,56,57,58]

Autoimmune disease is one of the common diseases that occur when the body's immune system turns against the body itself, attacking as if it were a foreign pathogen. Examples of autoimmune disorders include rheumatoid arthritis, multiple sclerosis, juvenile diabetes, cardiomyopathy, antiphospholipid syndrome, Guillain-Barre syndrome, Crohn's disease, Graves' disease, Sjogren's syndrome, alopecia, **myasthenia gravis**, lupus erythematous, and psoriasis.[39,59]

In addition, such disorders complicate other diseases that are not autoimmune in origin, such as atherosclerosis. A key challenge to developing therapies for autoimmune disorders is the cross-reactivity between normal body proteins and those of a variety of pathogenic agents. Therefore, it is important to allow as much independent function and self-care as possible and to promote appropriate activities to ensure a sense of normalcy in the body system.[60]

The development of modern biotechnology is characterized by intense development of biosafety guidelines when working on the genetic, cellular, tissue and organism level.

Today, about special attention is given to the criteria and indicators, biosafety, and in all countries of the world, there is a state legal regulation in various areas of biotechnology.

High biotechnology based on modification of genetic structures with the purpose of improvement of biological objects.

Management molecular and genetic events affecting indigenous mechanisms of the most important properties of living organisms, such as heredity, variability, energy and mass transfer, adaptation, resistance, productivity and production quality.

Molecular-genetic engineering and the effects of such interventions today carry to dual-use technology, as their results cannot always be predicted.

Artificial changes in mass and energy can also be related to changes in information characteristics of realization of genetic information. In society there is a certain caution in the use of genetically-modified plants, animals, microorganisms and viruses. And in some cases, lack of knowledge in this area can have a negative impact on the pace of development of biotechnology and especially in its strategic component, which is bioengineering. Today, experts in the field focused biotechnology are working to strengthen or weaken the virulence or other characteristics of bacteria, solving the problem of health safety and protection of the state against biological weapons and terrorism. It is necessary to take into account the relationship between the kinds of biosafety and the impact on their biotech production:[6]

1) In public health - in the production of medicines, vaccines, diagnostics, human reproduction, gene therapy and xenotransplantation;
2) When the production of new food products Modern biotechnology is aimed at the development of balanced food rations, the production of food products and dietary productive use of microbial producers for the production of bread, cheese, wine, food additives, enzymes;
3) Agriculture is important to obtain new transgenic plants and animals with desired productive, adaptive and autonomic properties and the development of biological protection of plants and animals and the creation of bacterial fertilizers;
4) In livestock - in vitro fertilization and cloning of embryos;

5) Providing a virus-free planting material for plants;
6) Establishment of recombinant microorganisms for the utilization of agricultural, domestic and industrial waste, for destruction of pollutants (pesticides, polymers, oil). Science, society and the State must develop and efficiently use the system of measures for the protection of human health and the environment from the harmful effects of hazardous factors.

It is necessary to develop a system of effective measures to combat and prevent the abusive use of microbiological science to achieve. The world scientific community and mainly specialists in the field of molecular biology have come to the conclusion that bioengineering is currently the only option for the efficient use of its results and achievements, and to address food security of the world population, diagnosis and treatment of diseases of civilization.

OBJECTIVES

We need to determine; - Viral persistence (which is a risk leading to infection occurrence) and immune-resistance (on the functional response of lymphocytes) in patients with different clinical phenotypes of myasthenia gravis.

The Task

1. To determine the presence of specific antibodies to the herpes virus in patients with different clinical phenotypes of myasthenia gravis.
2. Determine the ratio of subpopulations of blood cells as a potential reserve for the implementation of the immune response.
3. Identify potential mitogenic activity of serum components of blood of patients with different clinical phenotypes of myasthenia gravis.
4. Determine the proliferative potential of peripheral blood leukocytes of patients with different clinical phenotypes of myasthenia.
5. Biosafety measures to prevent bio-risks, contamination and invalid results in the diagnostic lab.

MATERIALS AND METHODS

LIST OF MATERIALS USED

a. Glass wares

The glass wares used are the conical flasks, cover slips, beakers, and disposable petri-dishes, glass rods, Nikiforov slide glass, measuring cylinder, and pipette, test tubes, and glass test bottles.

b. Equipment's

Autoclave, Bunsen burner, light microscope, thermostat, centrifuge, cell colony counter.

c. Reagents

Alcohol (95%), human blood, human plasma, penicillin, streptomycin, fetal calf serum, isotonic solution, heparin (anticoagulant), mitogen or PHA (phytohemagglutinin).

d. Media

Antibiotics: - penicillin and streptomycin, nutrient medium 199.

e. Other materials

Marker, sterile syringe, paper tape, Aluminum foil, cotton wool, vials, test tube rack, disinfectant, Microfluidic pipette.

METHOD

First Stage

1. Peripheral blood was taken aseptically and incubated for 30-40 minutes at room temperature until a visible clear separation of plasma and erythrocytes.
2. In sterile vials make 4 ml of nutrient medium 199 containing 200 units of penicillin and 100 units of streptomycin per 1 ml and 0.5 ml of inactivated fetal calf serum.
3. 0.3-0.5 ml of plasma containing cells was added in flasks with medium 199.
4. In the test sample was added 0.1 ml of an isotonic solution containing 100 mkg of PHA. In the control samples, 0.1 ml of isotonic solution was added (NO MITOGEN WAS ADDED).
5. Bottles are rightly placed for 48-72 hours in a thermostat at a temperature of 37digree Celsius (Room temperature).
6. After incubation, blasts were seen but they were in very small amount under the microscope.

Second Stage

7. Centrifuged for 10 min at 1000 rev / min, and the supernatant was removed (the excess liquid dispelled), and sediments were seen. After centrifugation, the solution was thoroughly mixed.

Fixation

8. 2ml of acetic acid and 6ml of methanol (1:3) were added to the sediments in the 2 test tubes (the test and the control). This mixture will help us in viewing the blast cells perfectly under the microscope. They were mixed thoroughly and kept for 10 minutes in an open place on a working bench while the fixation process is taking place.
9. Then the mixture undergoes centrifugation again for 10 minutes and no sediments are seen because of excess liquid. Therefore, excess liquid is dispelled again and the blast cells which are the residue at the bottom of the glass tubes.
10. The supernatant was re-mixed, and a quantity of 100 ml is removed with a pipette and smears are prepared on a sterile glass slide.
11. The slides are left to dry in a ventilation box for 72 hours.
12. Smear stained with azure-eosin by Romanowsky Giemsa for 30 min. Smear stained with azure-eosin by Romanowsky Giemsa for 30 min.
13. Microscopic view and cell count of blood cells (Lymphocytes and blasts).
14. Microscopic reading of dry drops of serum.

RESULTS AND DISCUSSION

The blasts and white blood cells were viewed under the microscope. White blood cells look smaller in size and blasts look bigger in size.

TEST

	WITH MITOGEN (PHA)	WITHOUT MITOGEN (PHA)
LYMPHOCYTES (%)	71.7%	62.5%
BLASTS (%)	28.3%	41.9%

CONTROL

	WITH MITOGEN (+ PHA)	WITHOUT MITOGEN (- PHA)
BLAST CONTROL	65%	10%

The blasts and white blood cells were viewed under the microscope. White blood cells look smaller in size and blasts look bigger in size.

It has been proven that under normal conditions 40 to 90 per cent of lymphocytes can transform into blasts. Decrease in blast transformation in the presence of allergens or PHA points to some abnormality. Therefore, we can say that there is a decreased functioning of the immune system. This could be as a result of different diseases such as inflammation, oncology diseases, and auto-immune diseases like Myasthenia gravis etc.

In addition, lymphocytes with mitogen have a spontaneous level of blast transformation. When mitogen is added there is an observable modulation in the action of different antigens. Therefore, mitogen helps the immune system for better functioning.[41, 42, and 43] Heparin helps to determine the osmotic resistance of erythrocytes in venous blood, it is also an anticoagulant.

Blast Index And Lymphocyte Index

PHA-Phytohaematogglutinin.

(+ PHA)- Proliferation of cell culture in the presence of mitogen.

(- PHA)- Spontaneous proliferation of cell culture without mitogen.

Blast index

$$\text{Blast index} = \frac{(+\text{PHA}) - (-\text{PHA}) \times 100}{(-\text{PHA})}$$

$$\text{I Blast} = \frac{(28.3\%) - (-41.9\%) \times 100}{(-41.9)\ \%}$$

$$\text{I} = \frac{70.2\% \times 100}{-41.9\%}$$

$$\text{I} = \simeq -167.54\%$$

Lymphocyte Index

$$\text{Lymphocyte index} = \frac{(+\text{PHA}) - (-\text{PHA})}{(-\text{PHA})} \times 100$$

$$I_{\text{Lymphocyte}} = \frac{(71.7\%) - (-62.5\%)}{(-62.5\%)} \times 100$$

$$I = \frac{134.2\%}{-62.5\%} \times 100$$

$$I \simeq -214.72\%$$

In a situation where we have (+ PHA) > (- PHA), there is a normal situation or normal healthy condition of the immune system of a person. And if reverse is the case, then there is a problem.

In the table above, in the blast transformation (+ PHA) < (- PHA) this proves the occurrence of a disease condition such as autoimmune disease (Myasthenia gravis). While in lymphocytes it is observed that (+ PHA) > (- PHA) and it shows the lymphocytes are in a normal condition.

Determination of Viral Persistence And Immune-Resistance (On The Functional Response Of Lymphocytes) In Patients With Different Myasthenia Clinical Phenotypes

Objective: To determine viral persistence and immune-resistance (on the functional response of lymphocytes) in patients with different myasthenia clinical phenotypes.

THE TASKS

1. **To determine the presence of specific antibodies to the herpes virus in patients with different myasthenia clinical phenotypes. (Table 1)**

Table 1: The concentration of IgG (mmol/l) antibodies to viral infection in patients with myasthenia on morphological and functional changes of thymus.

Index	Control group	Surveyed groups		
		Myasthenia without defeat in the thymus (M)	Myasthenia against the backdrop of thymic hyperplasia (MG)	Myasthenia against the backdrop of a thymoma (MT)
IgG against SMV	0,80 ± 0,05	22,8 ± 2,1 *	18,7 ± 3,7 *	23,1 ± 1,6 *
IgG antibodies to EBV	0,24 ± 0,03	2,01 ± 0,18 *	2,94 ± 0,09 *	2,36 ± 0,11 *

Note: * - $p<0.05$

Findings

1. The concentration of IgG antibodies to viral infection in patients with myasthenia on morphological and functional changes of thymus.
2. A high incidence of viral persistence could serve as a basis for the consideration of a viral infection as a trigger factor of the disease.
3. IgG against CMV (cytomegalovirus) showed the highest value when surveyed in **Myasthenia without defeat in the thymus** (M) and the lowest value when surveyed in **Myasthenia against the backdrop of thymic hyperplasia (MG)**.
4. IgG antibodies against EBV (Epstein-Barr virus) showed the highest value when surveyed in **Myasthenia against the backdrop of thymic hyperplasia** (MG) and the lowest value in **Myasthenia without defeat in the thymus (M).**

2. Determine the ratio of subpopulations of blood cells as a potential reserve for the implementation of the immune response. (Table 2)

Table 2: The differentiation of white blood cells (lymphocytes and the ratio neutrophils) in the patient groups with different clinical phenotypes of myasthenia gravis.

The clinical phenotype of myasthenia gravis	WBC counts			The ratio L / H * 100
	White blood cells, abs. X10[6]	Neutrophils %	Lymphocytes , %	
control				
M	6.07	59.5	25.2	42.35
MG	5.75	61.04	22.1	36.2
MT	6.68	65.65	29.05	44.24

Findings

1. It can be concluded that **lymphocytes** had the lowest count in the clinical phenotype of myasthenia gravis.
2. Also, **White blood cells** have the lowest count in the clinical phenotype of myasthenia gravis.

3. **Identify potential mitogenic activity of serum components of blood of patients with different clinical phenotypes of myasthenia gravis. (Table 3)**

Table 3: The proliferative activity of lymphocytes of patients with surgical pathology under the influence of serum enriched and depleted biopolymers.

	Proliferative activity of lymphocytes in cell culture		Stimulation Index
	Spontaneous	Stimulated	
The serum of patients with M	30	31.2	0.04
The serum of patients with MT	24.5	32.5	0.326

Findings

1. It has been shown that the components of the serum of patients with M did not significantly alter proliferative activity of lymphocyte in the control cultures.
2. The components of the serum of patients with myasthenia gravis in the background thymoma have a stimulating effect on proliferation.
3. The stimulation index in the first case was very low and amounted to 0.04, while the second was much higher and amounted to 0.326.

4. **Determine the proliferative potential of peripheral blood leukocytes of patients with different clinical phenotypes and the triggering factors of myasthenia gravis. (Table 4)**

Table 4 - The proliferative activity of lymphocytes in the patient groups with different clinical phenotypes of myasthenia gravis.

The clinical phenotype of myasthenia gravis	Proliferative activity of lymphocytes in cell culture		(F +) - (F-)	Stimulation Index
	Spontaneous	Stimulated with PHA		
control	8.0	35.0	27.0	3.37
M	16.3	54.6	38.3	2.34
MG	17.0	53.0	36.0	2.11
MT	24.0	51.2	27.2	1.13

Findings

1. It is shown that the spontaneous proliferative activity in patients with M much exceeds the reference value - 2 times or more and makes - 16.3%, and MG - 17.0% and the highest is MT and it is 24%.
2. In the presence of mitogen (PHA), proliferation of cells in culture in vitro with different clinical phenotypes of myasthenia gravis is higher than the control cell culture.

3. For a standardized results stimulation index was calculated, which compares the proliferative activity without PHA with proliferative activity with PHA. But the magnitude of the pre-proliferative activity with PHA subtracted the value of proliferative activity without PHA.
4. The stimulation index = (+ PHA) - (-PGA)/(-PGA) %

(+ PHA)- Proliferation of cell culture in the presence of mitogen in the cytoplasm.

(- PHA)- Spontaneous proliferation of cell culture without mitogen.

In recent years, there was a reversal of viral persistence. In patients with myasthenia detected with the population frequency of 95%, herpes viruses, Epstein-Barr virus and cytomegalovirus.

The possible error may occur: -

1. When drawing blood:

- Violation of the procedure of blood sampling;

2. When setting the method:

- Not enough clean dishes;
- The use of old or improperly prepared reagents;
- Bad staining.

Safety Measures And Disposal

Wear rubber gloves, lab coats, safety goggles. It is forbidden to eat, smoke.

All samples for analysis are considered potentially infectious material. Therefore, the remnants of samples and reagents must be disposed of in accordance with the established rules.

BLAST CELLS

Blast cells are immature cells found in bone marrow. They are not fully developed and therefore cannot carry out any function within the body yet. In normal humans, up to 5% of the cells found in the bone marrow are blats cells. When a higher percentage of them are found, further testing may be needed as this is an indication of one of several disorders which affect the blood and the bones. Normally, blast cells continue to mature within the bone marrow and then begin to carryout set functions. But when a higher than normal ratio of blast cell is found within the bone marrow, a problem may exist.[65,66,67]

Lymphocytes

Lymphocytes are small white blood cells that play a role in the body's immune response (i.e., in the body they fight against germs and diseases). There are two main types of Lymphocytes known as the B lymphocytes (B-cells) and the T lymphocytes (T cells).

- B cells- Produce antibodies that attack foreign molecules (germs and the toxins they produce).

T cells- They are more complicated, but they can attack the body's own cells when they are diseased. For example: - when the cells have been invaded by cancer or viruses.

DISCUSSION

1. The levels of biohazards in biotechnology research are divided into 4 classes. In order to reduce biological risks in establishments, modern biotechnology should strictly enforce biosecurity measures.
2. Big role in reducing biological risks of infectious diseases play a role in personal hygiene.
3. Viral infections can lead to the development of various pathologies, particularly in myasthenia. In myasthenia there can be a formation of different clinical phenotypes.
4. Development of myasthenia gravis is accompanied by a multiple increase in the content of immunoglobulin G, the change in the ratio of lymphocyte / neutrophil changes the proliferative activity of lymphocytes.[64,69,76]

CONCLUSION

Biotechnology is set to play a vital role in the future of the medical, agricultural, environmental, food pharmaceutical and industrial sectors of the world at large. The concern should be on how to tap into modern biotechnology, maximize the benefits of the technology and minimize the risks in terms of environmental harm and human health risks. New innovations in modern in modern biotechnology can as well serve as an eye-opener for biotechnologists to find a lasting solution to autoimmune diseases and other types of diseases now and in the future, thereby giving a better life and living standard to humans from generation to generation. Biosafety laws must be put in place to ensure products released are environmentally safe. Biosafety units should be set up at Institutions & Universities utilizing modern biotechnology. The public need to be educated on modern biotechnology.[74,75]

In addition, in the course of carrying out a blood test to determine the presence of an autoimmune disease or any other type of disease, very important biological safety must be

observed and strictly adhered to. This will enable valid results and avoid infections of any sort and risks in the laboratory as well as throughout the lab work.

Finally, it is important to allow as much independent function and self-care as possible and to promote appropriate activities to ensure a sense of normalcy in the body system.[21]

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81. Compare Chargaff, On the Dangers of Genetic Meddling, 192 SCIENCE 938 (June 4, 1976) with Cohen, Recombinant DNA: Fact and Fiction, 195 SCIENCE 654 (Feb. 18, 1977). See also 196 SCIENCE NO. 4286 (Apr. 8, 1977), which devotes an entire issue to recombinant DNA research.
82. See generally Subcommittee on Science, Research and Technology of the House Committee on Science and Technology, Genetic Engineering, Human Genetics, and Cell Biology: DNA Recombinant Molecule Research (Supplemental Report II) 32-42 (1976).
83. Berg, Baltimore, Brenner, Roblin & Singer, Summary Statement of the Asilomar Conference on Recombinant DNA Molecules, 72 PROC. NAT'L ACADEMY OF SCIENCES USA 1981 (1975).