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FEATURES OF IMMUNE STATUS AT PATIENTS BY ACUITY CORONAL SYNDROME UNDER HERPESVIRUS PERSISTENCE

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Atherosclerosis clinical presentation in whole and pathogenetically related to it coronary heart disease particularly are characterized not only by polymorphism of clinical implications but also by immune homeostasis disorders [7]. On the one hand, immune disorders play a certain role in atherosclerosis emergence, on the other hand, -atherosclerosis specifies autoimmune disorders of coronary vessels. The main group of atherosclerosis patients consists of middle age and senior persons. That's why the evaluation of immunological indicators in persons who suffer from coronary heart disease should be carried out taking into consideration the age peculiarities of the patient contingent. The immune system at this age has its own characteristics. During organism aging the principal function of the immune system that is the genetic homeostasis assurance, gradually diminishes.

"Nonshared autoantigens" recognition becomes less exact, immune reaction efficiency reduces. All this results in the emergence of immunological syndromes typical for this age such as immune deficiency, increase of circulating immune complex level, more frequent emergence of benign monoclonal gammopathies (hyperimmunoglobulenemia) [2,4]. Immunodeficiency consequence is effect reduction of immunization, antibody titer reduction in blood lower than protective level, weakening of cellular immune reactions.

Under aging T-lymphocyte system suffers most. It is evident in general number reduction of "adult" T-lymphocytes in blood, number reduction of T-helpers, synthesis of interleukin-2 and IgG and IgA class antibodies, phagocytic activity reduction of macrophages and neutrophils [8,13]. All these changes are specified by age involution of thymus after 50 years. The most important consequence of the immune system dysfunction at this age is sensitivity elevation to infections. Persistent herpesvirus infection itself evokes immunodeficiency and T-cellular component of immune system experiences the oppression the most.

Nowadays certain successes in study of atherogenesis different aspects are achieved, data about the role of immune mechanisms in atherosclerosis development and the effect of immunity factors on functional status of endothelium are received [3,4,13].

The works of Kiev researchers under the direction of I.M. Handzha showed the interconnection between persistent herpesvirus infection and changes taken place in endothelium against the background of alimentary hypercholesterinemia. The authors think it can be the result of control mechanisms disorder of T-system of immunity, the suppressor function of peripheral lymphocytes in particular [2].

With the beginning of virus reproduction in endotheliocytes, there observed quick enough infiltration by polymorphonuclear cells, lymphocytes and macrophages with lymphoquin secretion that must finally result in infected cell restriction and virus reproduction oppression [1,12,16].

It should be taken into account that herpes progresses under virus persistence during all life is the secondary immunodeficient disease [9]. Under the immune system imbalance of an organism that becomes older, the virus reproduction continues. It can have its clinical implication later. The role of immune mechanisms in atherosclerosis development is not doubtful at the present time [4,7]. Certain successes are achieved in the study of humoral and T-cell components of the immune response on cardiac hystiocyte damage that experience ischemization. The effect of immunocompetent cells on the functional status of endotheliocytes and other questions of immune homeostasis under atherogenesis are studied actively. The analysis of virus immunomodulatory action broadened the idea of researchers about pathogenesis of virus etiology disease and about the cytokine role in this process particularly [6,17,18]. The separate analysis of immunological changes under atherosclerosis and herpesvirus infection doesn't give an objective idea about the immune status of patients. There is a risk of subjectivism under such an approach to the analysis of macroorganism immune homeostasis. It is necessary to carry out a complex analysis of immune response in patients with atherosclerosis taking into consideration virus long-term persistence in the organism.

In this connection the purpose of our work is detection of possible interconnection between certain main indicators of immune homeostasis and persistent herpesvirus infection in patients with different forms of coronary heart disease (CHD).

Materials and methods

Patients with CHD who were undergoing treatment in infarction and rehabilitation departments of Kharkov clinical hospital № 8 were examined. The material for the research was blood taken from an ulnar vein in the morning, on an empty stomach.

The group of patients numbers 50 persons aged from 32 to 60. The average age was 52,6 ± 3,6 years. Among them there were 38 men and 12 women. The control group was composed of 26 persons conformable for sex and age with the experimental group but without clinical implications of CHD.

CHD clinical forms were stable angina (SA) – 13 patients, unstable angina (UA) represented by progressive exertional angina – 14 patients and acute myocardial infarction (MI) – 23 patients. The diagnosis was established on the basis of patient's complaints, the antecedent anamnesis, clinical, laboratory and tool research methods. A functional class of stable angina was defined according to the classification of the Canadian association of cardiologists.

For the immune status evaluation level-1 laboratory tests were carried out. They included calculation of lymphocyte general number, number determination of T and B-lymphocytes, evaluation of neutrophil phagocytic activity, determination in ELISA test serum immunoglobulin main classes: IgA, IgM, IgG and determination of complement titer [1,6].

Taking into account the fact that under latent herpesvirus infection mainly T-cellular component experiences changes and also the mean age of the examined there were carried out selectively such level-2 tests: determination of lymphocyte subpopulations (CD4+, CD8+), determination of circulating immune complexes (CIC) level, LBTR performance with phytohemagglutinin (PHA) for determination of lymphocyte functional status according to the standard methods [5,10,11].

The determination of herpes simplex virus and cytomegalovirus markers were carried out through fluorescent antibody method with the usage of monoclonal antibodies produced by "SANOFI DIAGNOSTICS PASTEUR", France, according to the given instructions. Statistical treatment of the research results was realized with the help of statistical application package Excel.

Results and discussions

The performed researches showed that in all examined clinical groups of CHD patients the oppression of T-cellular immunity component was observed (table 1).

T-lymphocyte number in different clinical groups of CHD patients is reduced in comparison with the control group. And their the least number was noted in the group of UA patients it was $(0,69 \pm 0,3)Ч10^9$/l, that is lower than the control indicator $(0,41 \pm 0,3) \times 10^9$/l.

Table 1. The main indicators of T-cellular component of the immune system in peripheral blood of CHD patients

CLINICAL GROUPS	**T-lym,** % (10^9/l)	**CD_4^+,** % (10^9/l)	**CD_8^+,** % (10^9/l)	**CD_4^+/CD_8^+**	**RBTL on PHA,** %
SA (n= 13)	43,7 ± 1,8∗ (0,75 ± 0,04)	20,2 ± 2,1∗ (0,23 ± 0,02)	12,1 ± 1,2 (0,15 ± 0,01)	1,53 ± 0,08∗	31,8 ± 4,1
UA (n= 14)	40,1 ± 1,7∗ (0,69 ± 0,05)	19,7 ± 2,0∗ (0,22 ± 0,02)	11,8 ± 1,5 (0,15 ± 0,01)	1,47 ± 0,09∗	22,1 ± 3,0∗
MI (n=23)	43,2 ± 1,8∗ (0,74 ± 0,03)	20,8 ± 1,6∗ (0,23 ± 0,03)	13,1 ± 1,3 (0,18 ± 0,02)	1,28 ± 0,04∗	20,9 ± 2,8∗
Control group (n= 26)	64,0 ± 2,5 (1,1 ± 0,04)	35,6 ± 2,4 (0,4 ± 0,03)	14,7 ± 1,5 (0,21 ± 0,02)	1,90 ± 0,02	46 ± 4,4

Note: ∗ - validity of discrepancy between patients' indicators and the control group ($p<0,05$)

In CD4+ and CD8+-T-lymphocyte populations the imbalance is observed. Reduction of CD4+ T-lymphocyte subpopulation reliably correlates with a blast transformation reduction of lymphocytes in the groups of patients with UA and MI. Literature information [5,9] indicates that the herpesvirus infection is accompanied by such a sharp reduction of CD4+ T-lymphocytes that evokes invert correlation of CD4+/ CD8+. The index reduction to 1,4 is considered as immunodeficiency indicator. The lower helper/suppressor ratio is, the stronger immunodeficiency is expressed. The obtained results confirm this data. Thereby the most apparent indicator reduction was observed under MI and UA. Under stable clinical course of angina helper/suppressor index approaches to critical value. Such a phenomenon can appear to be the consequence of several reasons. On the one part CD4+ subpopulation increase and CD8+ subpopulation reduction are characteristics of senior persons (older than 50), and on the other part – persistent herpesvirus infection can result in number imbalance of these immunocompetent cells. The classical example of which is herpes virus infection.

Pain syndrome and psychoemotional stress that accompanies CHD as a rule, are stress factors. The activation of anterior pituitary activity and the increase of steroid hormone secretion by adrenal glands are known to take place under inherently different stress factors that exceed physiological stimulus limits (I.A. Hrihorova 1997). The steroid hormone level in blood plasma correlates with the decrease of immunocompetent cell number and is accompanied by immunosuppressive effect. As a result the involution of lymphoid organs and lymphopenia are observed. According to Hans Selye the immune system reaction on stress factor is regarded as stereotype somatic detection of host defense mobilization. Under unstable angina and acute myocardial infarction through the peculiarities of their clinical detection, the adaptation to damaging agents activity is lower than under stable clinical course of CHD. It may be one of the reasons of more significant decrease of lymphocyte population in these two groups. However it should be noted that the immune response formation is influenced by factors connected with fundamental disease and possible concomitant pathology as well as with the age and sex of patients. In connection with a great number of immunogenic factors, often multidirectional, - changes in the immune status can be regarded as the result of complex multifactor influence with immune syndrome formation and possible tendencies of immune response [15].

In addition under immune status analysis the fact that immune system reaction on stimulation is always individual and genetically conditional should be taken into account. It is possible to get as many different responses on one stimulating factor as many people were observed. The immune response intensity is reduced if the influence of virus AG concurs with a stress factor. On the other part the integration of virus genetic material with immunocompetent cell gene can result in its functional incompetence.

Thus the determination of lymphocyte functional activity on T- mitogen in patients with CHD indicates the reduction of their transformation capability. Lymphocyte blast-cell transformation in response to phytohemagglutinin under all CHD clinical forms analyzed was considerably depressed. Under MI the level of blast-cell transformation was 20,9 % of the control level, under UA – 22,1 % and under SA – 31,8 %. The oppression of lymphocyte blast-cell transformation reaction is known to take place under exacerbation of recurrent herpesvirus infection. During remission the transformation of lymphocytes intensifies on the contrary. Among the examined patients with different forms of CHD there are antigens of CMV in 67,3 % and HSV in 53,3 %, though herpesvirus infection wasn't noted. Thereby a rise but not a reduction of blast transformation level could be expected. Perhaps the reason of low transformation capability of lymphocytes is advanced age of patients (52,6 ± 3,6) but on the other hand, the target organ under herpesvirus infection recurrence could be only vessel endothelium. In this case the development of intravascular inflammation syndrome could not be diagnosed because of the diagnostics complications in common clinical conditions.

The analysis of humoral immunity indicators shows the unreliable rise of B-lymphocyte number in all examined clinical groups of CHD patients in comparison with the control group data and the tendency to immunoglobulin hyperproduction (table 2).

Table 2. The principal indicators of B-cellular component of the immune system in patients with coronary heart disease

CLINICAL GROUP	**B-LYM,** % (10^3 kl./mcl)	**IgM,** g/l	**IgG,** g/l	**IgA,** g/l
SA (n = 13)	23,5 ± 1,1 (0,4 ± 0,02)	1,8 ± 0,2	14,0 ± 1,1	2,7 ± 0,2
UA (n = 14)	24,8 ± 1,7 (0,43 ± 0,02)	2,1 ± 0,18∗	16,8 ± 1,2∗	2,4 ± 0,2
MI (n = 23)	24,9 ± 1,3 (0,41 ± 0,03)	1,98 ± 0,1∗	15,5 ± 0,5∗	2,1 ± 0,3
Control group (n = 26)	23,0 ± 1,8 (0,4 ± 0,03)	1,5 ± 0,20	12,7 ± 1,2	2,3 ± 0,5

Note: ∗ - validity of discrepancy between patients' indicators of different clinical group and the control group($p<0,05$)

It should be noted that among immunoglobulin isotopes the rise of IgM and IgG levels is observed in UA and MI groups and in a less degree, as compared with the control group, in SA group. However, taking into consideration the middle age of the examined patients, IgA and IgG hyperproduction should have been expected. Perhaps the slight rise of IgM fraction in patients with CHD is connected with persistent virus infection recurrence. The rise of IgG level can be explained by the high level of infection by cytomegalovirus and herpes simplex virus type 1 and type 2of CHD patients. It is also confirmed by seroconversion (4-multiple titre increase of antibodies to CMV) in groups of patients with UA and MI.

It should be noted that there is no clear correlation of disease clinical course and severity of CHD with immune imbalance, but certain tendencies in immune status imbalance of UA and MI patients are still observed.

For more deep analysis of immune changes we calculated diagnostic value coefficients of every immunological indicator, which we determined by the formula proposed by A.D. Horelik and V.A. Skripkin [6]. Of all the obtained coefficients, we selected three with the lowest Kj value. The lower Kj coefficient was for a definite immune indicator, the more this indicator differed from the norm. The obtained immune disorder formulas (IDF) are given in table 3.

Thus, the obtained formulas of immune disorders under CHD confirmed the fact that the most significant immunologic changes are taken place in cellular component of immunity. The illustration of it is the reduction of T-lymphocyte population in all represented clinical groups. In the groups with stable angina and acute myocardial infarction the indicators of reduced functional activity of cellular component of immunity are on the second and third places. It is impossible to affirm that humoral immune response in these groups didn't experience certain changes. The increase of total CIC, IgM and IgG imbalance show the reaction from humoral component of immunity and perhaps the influence on fundamental disease pathogenesis. However, these changes are expressed in a less degree than the changes from cellular component.

Table 3. Immune disorder formulas in patients with different clinical forms of coronary heart disease

CLINICAL GROUPS	IMMUNE DISORDER FORMULAS
Stable angina	$T^- LBTR^- P^-$
Unstable angina	$T^- T_x^- IgG^+$
Acute Myocardial Infarction	$T^- T_c^+ LBTR^-$

References

1. Варгин В.В., Семенов Б.В. Вторичные иммунодефициты, возникающие в ходе инфекции // Иммунология инфекционного процесса. – 1993. – С. 178 – 192.
2. Ганджа І.М. До питання про патогенез атеросклерозу // Український кардіологічний журнал. – 1994. - №1. – С. 13 –16.
3. Гиріна О.М. Імунопатогенетичні та ендокринні зміни при різних формах ішемічної хвороби серця в умовах вірусної інфекції і їх терапевтична корекція: Автореф. дис. д-ра мед. наук. – К., 1996. – 45 с.
4. Дзяк Г.В., Коваль Е.А. Значение изменений иммунологического статуса в определении характера течения атеросклероза и ИБС // Журн. АМН України. – 1996. - Т.2, №2. – С. 317 – 334.
5. Исаков В.А., Борисова В.В., Исаков Д.В. Патогенез и лабораторная диагностика герпеса. Руководство для врачей. – Санкт-Петербург.: Лань. – 1999. – С. 197 -205.
6. Караулов А.В. Клиническая иммунология. –М.:МИА. – 1999.- С.93–121.
7. Малая Л.Т., Ладный А.И. Новые данные о клеточных и гуморальных факторах атерогенеза и их роль в гиполипидемической терапии // Журн. АМН України. - 1996. - №2. - С. 199 - 216.
8. Фрейдлин И.С. Иммунная система и ее дефекты. – Санкт-Петербург.: Лань – 1998. –110 с.
9. Фролов А.Ф. Персистенция вирусов. Механизмы и клинико-эпидемиологические аспекты. – Винница. Изд-во ВМУ им.Н.И.Пирогова. – 1995. – С. 150 – 171.
10. Хаитов Р.М., Пинегин Б.В. Вторичные иммунодефициты: клиника, диагностика, лечение // Иммунология. – 1999. - №1. – С.14 – 17.
11. Чумак А.А. Современные методы оценки лимфоцитов // Лабораторная диагностика. – 1998. - №2. – С. 28 - 31.
12. Bevilacqua, M. P. Endothelial leukocyte adhesion molecules // Annu. Rev. Immunol. - 1993. - № 11. – P. 767-804.
13. Fish, K., A. Depto, A. Moses, W. Britt, and J. Nelson. Growth kinetics of HCMV are altered in monocyte-derived macrophages // J. Virol. - 1995. - № 69. – P. 3737-3743.
14 Minton, E. J., C. Tysoe, J. H. Sinclair, and J. G. P. Sissons. Human cytomegalovirus infection of the monocyte/macrophage lineage in bone marrow // J. Virol. - 2000. - № 68. – P. 4017 - 4021.
15. Persoons, M. C. J., M. J. A. P. Daemen, J. H. Bruning, and C. A. Bruggeman. Active cytomegalovirus infection of arterial smooth muscle cells in immunocompromised rats: a clue to herpesvirus-associated atherogenesis? // Circ. Res. - 1994. - № 75. – P. 214 - 220.
16. Pollack, J. L., R. M. Presti, S. Paetzold, and H. W. Virgin. Latent murine cytomegalovirus infection in macrophages. // Virology. - 1997. - № 227. – P.168 -179.
17. Span, A., M. V. Dam-Mieras, W. Mullers, J. Endert, A. Muller, and C. Bruggeman. The effect of virus infection on the adherence of leukocytes or platelets to endothelial cells // Eur. J. Clin. Invest. - 1991. - № 21. – P. 331 - 333.
18. Taylor-Wiedeman, J., P. Sissons, and J. Sinclair. Induction of endogenous human cytomegalovirus gene expression after differentiation of monocytes from healthy carriers // J. Virol. - 1994. - № 68. – P. 1597 - 1604.

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ОСОБЕННОСТЫ ИММУННОГО СТАТУСА У БОЛЬНЫХ С ОСТРЫМ КОРОНАРНЫМ СИНДРОМОМ В УСЛОВИЯХ ГЕРПЕСВИРУСНОЙ ПЕРСИСТЕНЦИИ

Перемот С.Д., Смелянская М.В., Мартынов А.В., Перемот Я.А.

Иммунный статус больных ишемической болезнью сердца характеризуется дефицитом преимущественно Т-клеточного звена иммунитета, появлением функционально неполноценных Т-лимфоцитов, а также некоторыми изменениями гуморальных иммунных реакций. Выявляется взаимосвязь между Т-лимфопенией и фактом инфицированности герпесвирусами больных различными формами ИБС, что указывает на формирование синдрома вторичного иммунодефицита, который можно расценить как результат длительной персистенции герпесвирусов и проявление хронического внутрисосудистого воспаления, патогенетически связанного с атеросклерозом..

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ОСОБЛИВОСТІ ІМУННОГО СТАТУСУ У ХВОРИХ З ГОСТРИМ КОРОНАРНИМ СИНДРОМОМ В УМОВАХ ГЕРПЕСВІРУСНОЇ ПЕРСИСТЕНЦІЇ

Перемот С.Д., Смілянська М.В., Мартинов А.В., Перемот Я.О.

Імунний статус хворих на ішемічну хворобу серця характеризується дефіцитом переважно Т-клітинної ланки імунітету, появою функціонально неповноцінних незрілих Т-лімфоцитів, а також деякими змінами гуморальних імунних реакцій. Виявляється зв'язок між Т-лімфопенією і фактом інфікованості герпесвірусами хворих на різні форми ІХС, що вказує на формування синдрому вторинного імунодефіциту, котрий можно розцінювати як результат тривалої персистенції герпесвірусів і прояв хронічного внутрішньосудинного запалення, патогенетично пов'язаного з атеросклерозом.

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FEATURES OF IMMUNE STATUS AT PATIENTS BY ACUITY CORONAL SYNDROME UNDER HERPESVIRUS PERSISTENCE

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The immune status of the patients by ischemic heart diseases is characterized by deficiency of T-cells part of immune system, appearance of functionly incomplete T lymphocytes, and also some changes of humoral immune responses. The interrelation between a T-lymphopenia and fact herpesviral persistence for patients with various forms of IHD is taped, that specifies formation of a set of secondary immunodeficiency symptoms, which can be regarded as result of long herpesviral persistence and exhibiting of a chronic intravascular inflammation, pathogenetical linked with an atherosclerosis.