

Lymphogram features in patients with allergic reactions on the background of Epstein-Barr viral infection

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Abstract. Analyzed the results of lymphogram and activated lymphocyte markers in 62 patients with chronic Epstein-Barr virus infection (EBV) in various stages of activity. In 51,6% of patients with EBV infection detected in stage of replicative virus activity, in 25,0% of them – on the background of allergic symptoms. In these patients found a significant increase of T-helper and T-cytotoxic lymphocytes with activation of humoral immunity, installed increasing the number of activated and immunoregulatory lymphocytes.

Keywords: Epstein-Barr virus infection, lymphocytes, allergy syndrome, total serum IgE.

Introduction. Among chronic infections caused by the intracellular pathogens, herpes infections, particularly caused by the Epstein-Barr virus are of great anxiety among physicians. Nowadays according to numerous studies it was proved that association EBV with a number of cancers, lymphoproliferative, autoimmune diseases, chronic fatigue syndrome, secondary immunodeficiency, neurological syndromes and sore and atypical forms of diseases of various origins that are not amenable to traditional methods of treatment [1]. However, the relatively small number of messages is found on association EBV with allergic diseases, including atopic.

Overview of publications on the topic. There is evidence that infection with the virus in early childhood is a trigger of asthma and atopic dermatitis [5]. In the study of total serum IgE it was determined that the level of this immunoglobulin was lower in EBV seropositive persons between the ages of 6 and 29 months compared with older persons in which the results were opposite. This is explained by the fact that in the first years of life serum total IgE is low due to the age characteristics of the immune system [6]. According to other data, in experiments in vitro elevated levels of interleukin 4 (IL-4) promotes polyclonal activation EBV infected B-lymphocytes with switching synthesis immunoglobulin IgM, IgG, IgA to IgE by these cells immunoglobulin [8, 9]. There is evidence that in the pathogenesis of chronic EBV-infection the clonal expansion of EBV T-lymphocytes and natural killer cells (NK) plays the main part [7]. As a result of several studies in patients with primary lesions of these virus cells significantly higher levels of total serum IgE in the background relatively low titers of IgM, IgG are identified. Also in these individuals hyperergic reaction to mosquito bites was observed [4]. According to V. Chopyak et al. (2010) it was found that patients with chronic EBV-infection in the stage reactivation combined immunodeficiency infringement by lymphocyte-phagocytic type, complications in 18,0% of hyper-IgE-dependent syndrome were revealed. Some people mainly with affected nervous system the level of serum IgE was more than 3000 IU/mL often without obvious clinical manifestations of allergic reactions in history [2].

Aim. The aim of our study was to investigate peculiarities of phenotypic characteristics of lymphocytes and their activated markers in patients with chronic persistent of EBV-infection in the stage of viral replicative activity with clinical and laboratory

manifestations of allergic syndrome.

Materials and methods. 62 individuals treated in the outpatient department and supervised at Lviv Regional Medical Center of Clinical Immunology and Allergology during 2013 were observed. The age of patients was examined 22,6±2,4 years, among them 33 (53,3%) women and 29 (46,7%) men. All patients were carried out clinical, instrumental and laboratory and special immunological studies.

A comprehensive diagnostic study of serum was conducted to determinate EBV serological markers (EBV-VCA-IgM/IgG, EBV-EBNA-IgG) with the help of indirect two-phase chemiluminescence immunoassay (CLIA) on the test-systems «DiaSorin» (Italy) using the analyzer «LiaiSon». Determination of DNA EBV in blood, saliva and mucous of posterior pharyngeal wall was performed with the help of polymerase chain reaction (PCR) on the diagnosticums «AmpliSens» (Russia) using «Rotor Geen 6000» (Corbett Research, Australia). Phenotyping of lymphocytes and determination of the expression of activation markers was performed using monoclonal antibodies on the cytofluorometer «Bekton Dickenson» (USA).

The results of the research were analyzed using the method of variation statistics using STATISTICA 6 program (Statsoft, USA). The control group consisted of 20 healthy individuals of appropriate age and gen.

Results and discussion. Based on the results of PCR in the study of saliva, blood and mucus of posterior pharyngeal wall it was revealed that DNA-virus was not detected in 30 individuals (48,4%). Based on the presence of EBNA-IgG+ and low titers of specific EVV-VCA-IgG+ compared with the control group, diagnosis of chronic EBV-infection was verified in these individuals in the latent stage.

In the other 32 (51,6%) patients DNA EBV "+" was detected, 13 (40,7%) patients – in mucosa of posterior pharyngeal wall, 6 (18,7%) patients – in saliva, 5 (15,6%) patients – in the blood, 5 (15,6%) patients – both in blood and mucosa of posterior pharyngeal wall, 3 (9,4%) patients – in three biological environments at the same time. DNA EBV "+" (52,0%) was more often found in association with the DNA-virus of human herpes of type 6, rarely (8,0%) – in association with cytomegalovirus, in 5,0% of patients – with viruses of simple herpes of types 1 and 2. In 3,0 % of cases DNA EBV "+" was determined to be affected by three above mentioned pathogens.

Based on the identified DNA EBV "+" and increased in 5-10 times compared with the control group titers of EBV-VCA-IgG+ and EBNA-IgG+ patients were diagnosed EBV chronic infection in the stage of replicative activity. Results of detailed historical information and clinical investigations showed that the recurrence of chronic EBV infection were accompanied with the following clinical manifestations: chronic fatigue syndrome – in 29 (87,5%) patients, infectious immunodeficiency syndrome – in 18 (56,3%) patients, syndrome of long subfebrile – in 18 (56,3%) patients, lymphadenopathy syndrome – in 15 (46,9%) patients, allergy syndrome – in 8 (25,0%) patients, autoimmune syndrome – in 7 (21,8%) patients and neurological syndrome – in 5 (15,6%) patients.

Due to the fact that we were more interested in patients with clinical manifestations of allergy syndrome, we

identified the appropriate group of patients (8 people) and conducted additional laboratory and specific immunological research.

Verification of allergy syndrome was carried out according to the following clinical features: in six (75,0%) patients were diagnosed maculo-papular rash on the skin of the upper limbs and upper chest with itching, and four (50,0%) patients complained on recurrent sneezing on background of liquid mucous discharge from the nasal cavity and tears, and bouts of dry cough were observed in three (37,5%) patients. When smears of nasal mucosa had been carried out in patients with catarrhal symptoms, increasing of the number of eosinophils (from 17% to 65%) was found. Investigation of total serum IgE showed an increase of this index within 119,5 – 325,6 IU/ml in six (75,0%) patients.

Table 1. Indices of lymph gram and activation markers of lymphocytes in patients with chronic EBV-infection in the phase of virus replication, (M±m)

| Indices | Healthy | First group, DNA(-) | Second group, DNA(+), allergic |
|---------------------|------------|---------------------|--------------------------------|
| | n=20 | n=30 | n=8 |
| Leucocytes, g/l | 6,53±0,26 | 5,12±0,15* | 6,62±1,46 |
| Lymphs (CD45+), % | 34,40±1,87 | 34,08±1,43 | 31,31±1,52 |
| Lymphs (CD45+), g/l | 2,22±0,15 | 1,71±0,07* | 2,05±0,5# |
| CD3+, % | 72,90±1,24 | 62,75±1,19* | 68,0±1,20 |
| CD3+, g/l | 1,61±0,10 | 1,06±0,03* | 1,39±0,17# |
| CD4+, % | 48,60±1,50 | 40,58±1,24* | 46,0±1,12# |
| CD4+, g/l | 0,78±0,04 | 0,68±0,02* | 0,94 ±0,22#* |
| CD8+, % | 24,30±1,01 | 20,17±1,12* | 18,0±1,63* |
| CD8+, g/l | 0,37±0,03 | 0,34±0,22 | 0,35±0,06 |
| CD16+/56+, % | 11,10±0,15 | 17,42±0,62* | 8,08±1,19# |
| CD16+/56+, g/l | 0,24±0,02 | 0,29±0,01* | 0,17±0,08# |
| CD19+, % | 9,60±0,61 | 10,00±0,83 | 12,10±1,15* |
| CD19+, g/l | 0,21±0,09 | 0,18±0,08 | 0,25±0,06# |
| IRI Abs Cnt | 2,04 | 2,05 | 3,83#* |
| CD3+/CDHLA-DR+, % | 10,70±1,9 | 7,10 ±0,7* | 11,24±0,92# |
| CD3+/CDHLA-DR+, g/l | 0,16±0,02 | 0,07±0,01* | 0,15±0,03# |
| CD4+/CD25+, % | 10,50±0,40 | 11,00±0,58 | 17,21±1,39#* |
| CD4+/CD25+, g/l | 0,09±0,01 | 0,07±0,01 | 0,17±0,03#* |

Remark: 1) * – possible difference of indices in patients of the first and second groups with 3 control one (p<0.05);
2) # – possible difference in patients of the first and second groups (p<0.05).

Thus, on the basis of the conducted research three groups of people were singled out: the control group (healthy), 20 people, the first group – patients with DNA EBV "-" 30 people, the second group – patients with DNA EBV "+" and 8 people with manifestations of allergy syndrome.

In these groups, a study of population and subpopulation composition of lymphocytes and their activation markers was conducted (Table 1). As shown in Table 1, in patients with chronic EBV-infection in latency period it was observed likely reduction of the absolute number of leukocytes (5,12±0,15 g/l, p<0,05) and lymphocytes (1,71±0,07 g/l, p<0,05) compared with the control group (respectively 6,53±0,26 g/l; 2,22±0,15 g/l). In general, studies lymphogram of patients of the 1st group indicated the formation of a special

immunopathological complex with features of deficiency of T-cell part of the immune system. This was confirmed by the likely decrease of absolute (1,06±0,03 g/l) and relative (62,75±1,19%) indices of CD3+-lymphocytes subsets compared with healthy individuals (respectively 1,61±0,10 g/l; 72,90±1,24%, p<0,05). In patients of the 1st group it was defined the reduction of the number of CD8+-lymphocytes possessing suppressor-cytotoxic effect (20,17±1,12%, p<0,05) and a significant reduction in the absolute (0,68±0,02 g/l, p<0,05) and relative (40,58±1,24%, p<0,05) the number of CD4+-lymphocytes compared with healthy individuals (respectively 24,30±1,01%; 0,78±0,04 g/l). The relative number of CD16+/56+ cells was significantly higher (17,42±0,62%, p<0,05) compared with control group (11,10±0,15%), which probably indicates the activation of antiviral parts

of the immune system. Regarding to absolute figures of CD19+ lymphocytes, the number was also lower ($0,18 \pm 0,08$ g/l) compared with controls ($0,21 \pm 0,09$, g/l $p < 0,05$). The absolute number of lymphocyte activation markers CD3+/CDHLA-DR+ was 2.3 times lower ($p < 0,05$) than in the control group and patients in the 2nd group, which may indicate minimization of inflammatory reactions at the site of persistence of EBV. The absolute number of CD4+/CD25+-regulatory lymphocytes has only a downward trend in comparison with healthy people and was lower ($p < 0,05$) compared to the 2nd group. Thus, in patients with chronic persistence of EBV in latent stage, we observed a decrease of T-lymphocytes by both T-helper and T-cytotoxic lymphocytes, which allows to confirm the presence of acquired/secondary immunodeficiency by lymphocytic type of virus origin in these patients. Increase in the number of NK-cells probably indicates the presence of EBV in the body, resulting in activation of antiviral innate cellular mechanisms. Reduction of the number of activated CD3+/CDHLA-DR+- lymphocytes indicates the absence of inflammation, that is proved by the same number of regulatory lymphocytes (CD4+/CD25+).

As for the 2nd group of patients with recurrent chronic EBV- infection and manifestations of allergy syndrome, the results of lymphogram had its own peculiarities. The first thing that attracted the attention was the fact that the absolute levels of white blood cells did not differ significantly from those in healthy subjects and patients of the 1st group. The absolute number of lymphocytes in the patients in the 2d group was significantly higher than in patients of the 1st group ($2,05 \pm 0,5$ g/l and $1,71 \pm 0,07$ g/l, $p < 0,05$). The absolute number of CD3+-lymphocytes did not differ significantly from that of healthy individuals, but was significantly higher than in patients of the 1st group (respectively $1,39 \pm 0,17$ g/l and $1,06 \pm 0,03$ g/l, $p < 0,05$). If the number of T-cytotoxic lymphocytes in patients of the 2nd group did not differ significantly from that of healthy individuals and patients of the 1st group, the number of T-helper cells in these patients was significantly higher and amounted to $46,0 \pm 1,12\%$, $0,94 \pm 0,22$ ($p < 0,05$). According to these figures IRI increased to 3,83, which was significantly higher compared with patients of the 1st group (2,05) and healthy individuals (2,04). The number of NK-cells in the test group patients was significantly lower ($8,08 \pm 1,19\%$) from that of healthy individuals and patients of the 1st group (respectively $11,10 \pm 0,15\%$ and $17,42 \pm 0,62\%$, $p < 0,05$). As for B-cells, their absolute number was significantly higher ($0,25 \pm 0,06$ g/l) compared with patients of the 1st group ($0,18 \pm 0,08$ g/l, $p < 0,05$). The number of activated T- and B-lymphocytes in patients of the 2nd group remained stable and did not significantly differ from the patients of the 1st group and healthy individuals. The number of CD4+/CD25+-lymphocytes in patients of the 2nd group was significantly higher than in the control group and patients of the 2nd group, both in absolute and in relative terms (respectively $0,17 \pm 0,03$ g/l and $17,21 \pm 1,39\%$, $p < 0,05$).

As for 2nd group patients with recurrent chronic EBV-infections and allergic syndrome, had their own peculiarities of the results of myelogram. The first thing that attracted attention was that the absolute levels of

white blood cells did not differ significantly from those of healthy individuals and patients of the 1st group. The absolute number of lymphocytes in the patients of 2nd group was significantly higher than in patients of 1st group ($2,05 \pm 0,5$ g/l and $1,71 \pm 0,07$ g/dL, $p < 0,05$). The absolute number of CD3+-lymphocytes in patients of 2nd group did not differ significantly from that in healthy individuals, however, was significantly higher than in patients of 1st group (respectively $1,39 \pm 0,17$ g/l and $1,06 \pm 0,03$ g/l, $p < 0,05$). If the number of cytotoxic T-lymphocytes in the patients 2nd group did not differ significantly from that of healthy individuals and patients 1st group, the number of T-helper cells in these patients was significantly higher and amounted to $46,0 \pm 1,12\%$, $0,94 \pm 0,22$ ($p < 0,05$). According to these indicators IRI increased to 3,83, which was significantly higher in comparison with patients of 1st group (2,05) and healthy individuals (2,04). Number of NK-cells in a test group of patients was significantly lower ($8,08 \pm 1,19\%$) from that of healthy individuals and patients of 1st group (respectively $11,10 \pm 0,15\%$ and $17,42 \pm 0,62\%$, $p < 0,05$). As for the B-lymphocytes, their absolute number was significantly higher in patients of 2nd group ($0,25 \pm 0,06$ g/l) in comparison with patients of 1st group ($0,18 \pm 0,08$ g/l, $p < 0,05$). The number of activated T and B lymphocytes in the patients of 2nd group remained stable and was not significantly different from the data of healthy individuals, and was higher than in patients of 1st group ($p < 0,05$). Number of CD4+/CD25+-lymphocytes in these patients was significantly higher than in the control group and patients in 1st group, both in absolute and in relative terms (respectively $0,17 \pm 0,03$ g/l and $17,21 \pm 1,39\%$, $p < 0,05$).

Thus, in patients with chronic EBV-infection with replicative activity of the virus and allergic IgE-dependent syndrome on the background of the normal number of lymphocytes and T-lymphocytes significant increase in T-helper cells and decrease in T-cytotoxic lymphocytes was observed, which greatly deepened the deficiency of specific antiviral defense. Reduction of the number of NK-cells enhanced the antiviral deficit. The increase in relative and absolute numbers of B-lymphocytes probably contributed to the activation of these cells according to the production of IgE, which correlated with its level in the blood of patients of the 2-nd group.

The inflammatory process in patients with allergic syndrome initiates not only IgE, but also activated T- and B-lymphocytes, as indicated by an increase in the number of CD3+/CDHLA-DR-lymphocytes. The increase in CD4+/CD25+-lymphocytes from one hand minimizes target lesions (first of all – local), on the other hand slows the elimination of the virus from the body by reducing the inflammatory immune response to EBV process [3].

Summary.

1. Allergic syndrome in patients with chronic EBV-infection in the stage replicative activity (DNA EBV"+") verified in 25,0% of patients.

2. In patients with allergic syndrome on the background of EBV-infection in the stage of replicative viral activity significant increase in T-helper cells and decrease in T-cytotoxic lymphocytes with activation of humoral branch, an increased number of activated and immunoregulatory lymphocytes was observed, that

enhanced the inflammatory process and deficiency of antiviral mechanisms.

3. In patients with allergic syndrome on the background of EBV-infection in the stage of replicative activity the following clinical manifestations: maculopapular rash, allergic rhinosinusitis, conjunctivitis, bronchospasm were recorded.

4. In 75,0% of patients with allergic syndrome on the background EBV- infection in the stage of replicative activity an increase in total serum IgE was revealed.

5. In the scheme of treatment of such patients therapy foreseen in the acts of the relevant allergic diseases must be used.

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Особенности лимфограммы у больных с аллергическими проявлениями на фоне Эпштейна-Барр вирусной инфекции

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Аннотация. Проанализированы результаты лимфограммы и активированных маркеров лимфоцитов у 62 пациентов с хронической Эпштейна-Барр вирусной инфекцией (EBV) в разных стадиях активности. У 51,6% больных выявлена ВЭБ инфекция в стадии репликативной активности вируса, у 25,0% из них – на фоне аллергической симптоматики. У данных пациентов выявлено значительное увеличение Т-хелперов и Т-цитотоксических лимфоцитов с активацией гуморального звена иммунитета, установлено увеличение числа активированных и иммунорегуляторных лимфоцитов.

Ключевые слова: Эпштейна-Барр вирусная инфекция, лимфоциты, аллергический синдром, общий сывороточный IgE.