ORIGINAL ARTICLE, MEDICINE

Immune Cell Subsets Evaluation as a Predictive Tool for Hepatitis B Infection Outcome and Treatment Responsiveness

Snezhina M. Kandilarova¹, Atanaska I. Georgieva¹, Anastasiya P. Mihaylova¹, Marta P. Baleva¹,
Valentina K. Atanasova¹, Diana V. Petrova², Georgi T. Popov³, Elissaveta J. Naumova¹

¹ Department of Clinical Immunology and Stem Cell Bank, Alexandrovska University Hospital, Medical University of Sofia, Sofia, Bulgaria

² Clinic of Propaedeutics of Internal Diseases, Alexandrovska University Hospital, Medical University of Sofia, Sofia, Bulgaria

³ Clinic of Infectious Diseases, Military Medical Academy, Sofia, Bulgaria

Correspondence:

Snezhina M. Kandilarova, Department of Clinical Immunology and Stem Cell Bank, Alexandrovska University Hospital, Medical University of Sofia, 1 G. Sofiyskiy St.

1431 Sofia, Bulgaria

E-mail: sneji_jm@yahoo.com

Tel: +359 2 9230690

Received: 11 Sep 2015

Accepted: 31 Aug 2016

Published Online: 29 Nov 2016

Published: 27 March 2017

Key words: HBV infection, cellular immune response, immunophenotyping

Citation: Kandilarova SM, Georgieva AI, Mihaylova AP, Baleva MP, Atanasova VK, Petrova DV, Popov GT, Naumova EJ. Immune cell subsets evaluation as a predictive tool for hepatitis B infection outcome and treatment responsiveness.

Folia Medica 2017;59(1):53-62.

doi: 10.1515/folmed-2017-0008

Background: The patient's immune response is one of the major factors influencing HBV eradication or chronification, and it is thought to be responsible for the treatment success.

Aim: Our study aimed to investigate whether cellular defense mechanisms are associated with the course of HBV infection (spontaneous recovery [SR] or chronification [CHB]) and with the therapeutic approach.

Patients and methods: A total of 139 patients (118 with CHB, 21 SR) and 29 healthy individuals (HI) were immunophenotyped by flowcytometry. Fifty-six patients were treatment-naïve, 20 were treated with interferons and 42 with nucleoside/nucleotide analogues.

Results: Deficiency of T lymphocytes, helper-inducer (CD3+CD4+), suppressor-cytotoxic (CD8+CD3+) and cytotoxic (CD8+CD11b-, CD8+CD28+) subsets, activated T cells (CD3+HLA-DR+, CD8+CD38+) and increased CD57+CD8- cells, elevated percentages of B lymphocytes and NKT cells were observed in CHB patients compared with HI. In SR patients, elevated CD8+CD11b+, NKT and activated T cells were found in comparison with controls. The higher values of T cells and their subsets in SR patients than in CHB patients reflect a recovery of cellular immunity in resolved HBV infection individuals. In both groups of treated patients, reduced T lymphocytes, CD3+CD4+ and CD8+CD38+ subsets were found in comparison with HI. Higher proportions of cytotoxic subsets were observed in treated patients compared with treatment-naïve CHB patients, more pronounced in the group with interferon therapy.

Conclusion: Our data demonstrate that cellular immune profiles may be of prognostic value in predicting the clinical course of HBV infection, and the determination of the therapeutic response.

BACKGROUND

Infection with hepatitis virus B (HBV) represents a global healthcare and social problem. An estimated 240 million people are chronically infected with hepatitis B (defined as hepatitis B surface antigen positive for at least 6 months).¹ The incidence of HBV infection in the general Bulgarian population is as high as 5%, which places Bulgaria among the regions in Europe with high prevalence of the infection. However, in comparison with the global prevalence rates according to recent reports it is in the lower-intermediate endemicity regions (2-4.99%).²

The immunopathogenesis of chronic HBV infection is not fully understood. After HBV contamination

both innate and adaptive immune responses are triggered to overcome the virus and ultimately terminate the infection. It is generally acknowledged that the host B-cell response (anti-HBV antibodies) contributes to the virus clearance and prevents viral spread while the cellular immune response is the most important factor in killing infected cells and is thought to be responsible for both viral clearance and disease pathogenesis during hepatitis B virus infection.³ In chronic infection, the severity of liver involvement is thought to depend on many factors including the host immune response, the virus itself (e.g. viral genome and mutants) and the environmental influence. Any changes or

dysfunction of the cellular immunity seem to be crucial for anti-HBV defense and thus for defining which individual will develop a chronic infection and which one will spontaneously clear the virus. The anti-viral treatment is also very important for disease outcome but its impact on the cellular immune response is not extensively studied.

AIM

To investigate whether the cellular immune response may be associated with the course of HBV infection (spontaneous recovery or chronification) and with the type of therapy chosen: interferons or nucleoside/nucleotide analogues.

PATIENTS AND METHODS

MATERIALS

A total of 139 patients were enrolled in the study. These included 118 patients with chronic hepatitis B (84 men, 34 women, mean age 44 ± 27 yrs; range 18-73 yrs) and 21 spontaneously recovered (SR) patients from acute infection (11 men, 10 women, mean age 44 ± 13.5 yrs; range 31-58 yrs). The recruitment of patients for the study was carried out in the Department of Gastroenterology at the Clinic of Propaedeutics of Internal Diseases, Alexandrovska University Hospital and Clinic of Infectious Diseases, Military Medical Academy, Sofia after clinical and laboratory evaluation according to predefined inclusion and exclusion criteria. The diagnosis of chronic hepatitis B (CHB) infection was based on the recommendations of the American Association for the Study of Liver Diseases. Co-morbidities, including HCV and HDV hepatitis, as well as other infections, tumors, autoimmune and inflammatory conditions were excluded. Fifty-six patients were newly diagnosed and treatment-naïve at the time of the study. Twenty patients were treated with interferons - IFN (Peg interferon α -2a) and 42 with nucleoside/nucleotide analogues (NA). The survey included 29 randomly selected unrelated healthy subjects from the general Bulgarian population, age- and sex-matched with the study patients, without a family history of inherited diseases, with normal liver function and negative serological markers for viral hepatitis. Clinical and laboratory signs of infection potentially influencing the immune cell populations were not detected in the controls. Healthy subjects were included to assess whether there were immune profiles in patients with hepatitis B associated with impaired defense mechanisms resulting in aggrava-

tion of the disease. All enrolled patients and healthy subjects signed informed consent.

METHODS

Investigation of immune cells

The cellular immunity was determined by immunophenotyping of peripheral blood cells using a panel of monoclonal antibodies conjugated with different fluorochromes. Samples acquisition and analyzes were performed on a flow cytometer (FACSCanto) using FACSDiva (Becton Dickinson) software. The following immune cell populations were determined by multicolor flowcytometry: total T (CD3+) lymphocytes and their two subpopulations – helper-inducer (CD3+CD4+) and suppressor-cytotoxic (CD3+CD8+) cells, B (CD19+) cells and NK [CD3-(CD16+56)+] cells; cell subsets with putative cytotoxic (CD8+CD11b-) and suppressor (CD8+CD11b+) activity^{4,5}; CD8+CD28+ T cells with effector (cytotoxic) and/or memory function⁶; terminally differentiated cell subpopulation with probable memory/effector function [CD8+CD28-; CD8+CD57+]⁶⁻⁸; CD57+CD8- cells with NK cells activity⁹; activated T cells [CD3+HLA-DR+, CD8+HLA-DR+ and CD8+CD38+]¹⁰; T lymphocytes with NK activity [NKT – CD3+(CD16+56)+].

STATISTICS

Data from the different investigated groups were compared using Mann-Whitney U test and SPSS v.16 statistical software package. The results were presented as mean value $\pm \sigma$. P values < 0.05 were considered statistically significant.

RESULTS

As a first step we examined the cellular immune response in HBV-infected patients and control group. Comparing the group of CHB patients with healthy individuals we found the following statistically significant differences (**Table 1**): deficiency of total CD3+ T lymphocytes due to a reduction of their helper-inducer, suppressor-cytotoxic and cytotoxic (CD8+CD11b-; CD8+CD28+) subsets; increased CD57+CD8- cells with NK cell activity and decreased activated total T cells (CD3+HLA-DR+) and CD8+ lymphocytes expressing CD38 activation marker (CD8+CD38+); elevated percentages of B lymphocytes and NKT [CD3+(CD16+56)+] cells.

In patients spontaneously recovered from HBV infection we found the following statistically significant differences in comparison with the healthy subjects: elevated CD8+CD11b+ cells (including T

Table 1. Cell subsets in patients with chronic hepatitis B, spontaneously recovered from HBV infection and healthy individuals

Cell populations	Chronic hepatitis B (CHB)	Spontaneously recovered (SR)	Healthy	P		
	(n=56) %/abs. number*	(n=21) %/abs. number*	(n=29) %/abs. number*	CHB / Healthy	SR / Healthy	CHB / SR
T cells (CD3+)	67.43±7.54 1143±560	72.48±8.44 1796±588	74.51±6.31 1512±439	0.000 0.000	0.561 0.070	0.007 0.000
Activated T cells (CD3+HLA-DR+)	5.98±4.01 99±73	14±8.07 343±226	9.00±4.74 166±65	0.015 0.001	0.048 0.001	0.000 0.000
Helper/inducer T cells (CD3+CD4+)	38.45±7.76 658±342	42.71±8.06 1060±387	46.08±6.64 965±313	0.000 0.000	0.23 0.381	0.020 0.000
Suppressor/cytotoxic T cells (CD3+CD8+)	25.57±7.38 439±237	29.14±9.12 726±334	29.76±5.97 624±167	0.000 0.009	0.314 0.431	0.177 0.000
CD4/CD8 ratio	1.72±0.94	1.7±0.58	1.65±0.48	>0.05	>0.05	0.619
B cells (CD19+)	12.95±4.75 226±6.08	10.67±4.48 269±100	9.24±2.81 192±82	0.000 0.347	0.231 0.009	0.064 0.054
NK cells [CD3-(CD16+56)+]	16.07±6.08 263±143.0	14.81±8.1 360±232	14.00±6.41 289±149	0.144 0.445	0.925 0.360	0.192 0.055
NKT cells [CD3+(CD16+56)+]	9.3±5.21 143±84	11.9±4.4 286±119	6.56±4.68 137±113	0.012 0.442	0.000 0.000	0.015 0.000
CD57+CD8- cells	11.71±6.94 206±159	8.78±6.14 212±151	5.30±3.77 115±99	0.000 0.004	0.023 0.013	0.097 0.745
CD57+CD8+ cells	14.76±6.1 254±188	13.28±5.62 320±185	12.74±6.20 267±151	0.235 0.423	0.804 0.437	0.334 0.089
CD8+CD11b+ cells	14.46±6.36 242±136	21.77±8.3 538±270	10.71±4.09 220±104	0.009 0.755	0.000 0.000	0.000 0.000
CD8+CD11b-cells	15.41±5.78 260±141	13.9±4.0 347±155	19.30±6.10 388±138	0.022 0.002	0.009 0.341	0.350 0.037
CD8+CD28+ cells	13.45±3.93 228±107	18.43±6.5 450±214	17.34±3.80 340±115	0.002 0.003	0.561 0.104	0.001 0.000
CD8+CD28- cells	16.06±6.36 270±189	16.67±7.02 372±201	12.58±5.89 245±141	0.078 0.824	0.089 0.039	0.768 0.012
CD8+CD38+ cells	17.22±7.64 267±149	27.43±7.71 684±288	21.70±7.04 425±123	0.028 0.001	0.040 0.001	0.000 0.000
Activated CD8+HLA-DR+ cells	5.1±3.26 83±66.0	4.2±2.87 107±92	6.78±4.14 132±70	0.101 0.008	0.061 0.112	0.333 0.314

* cells/ μ l

lymphocytes with suppressor function), NKT lymphocytes, CD57+CD8- NK cells, activated total T lymphocytes (CD3+HLA-DR+) and CD8+CD38+ cell subset.

The comparative analysis between both patients' groups (**Table 1**) showed statistically significantly higher values of total T cells and their helper-inducer (CD3+CD4+), suppressor-cytotoxic (CD8+CD3+) and effector (cytotoxic) and/or memory subpopulations (CD8+CD28+) in SR versus CHB patients which is due to normalization of these immuno-

logical parameters in the individuals who have resolved their HBV infection (**Fig. 1**). We also observed elevated activated T cells (CD3+HLA-DR+, CD8+CD38+) and NKT cells in patients recovered from the infection.

In order to assess whether treatment may have impact on the cellular immune response of chronically infected HBV individuals (**Table 2**) we made a comparison between both patients' groups under therapy (treated with IFN and treated with NA), and between treated and untreated patients and

Table 2. Cell subsets in treatment naive (newly diagnosed) and treated with antiviral drugs patients with chronic hepatitis B patients, and in healthy controls

Cell population	Chronic hepatitis B (CHB)			Healthy (n=29)	P	
				%/abs. number*		
	Patients treated with interferon IFN (n=20)	Patients treated with nucleoside/ nucleotide ana- logues NA (n=42)	Non-treated patients (n=56) NT		IFN/ healthy	NA / healthy
	%/abs. number*	%/abs. number*	%/abs. number*			
T cells (CD3+)	70.05±6.12 1028±441	68.33±6.18 1163±508	67.43±7.54 1143±560	74.51±6.31 1512±439	0.000 0.018	0.002 0.000
Activated T cells (CD3+HLA-DR+)	8.45±7.18 122±104	6.48±4.06 103±67	5.98±4.01 99±73	9.00±4.74 166±65	0.377 0.046	0.046 0.003
Helper/inducer T cells (CD3+CD4+)	39.35±9.0 558±230	38.21±7.08 660±330	38.45±7.76 658±342	46.08±6.64 965±313	0.011 0.000	0.000 0.000
Suppressor/ cytotoxic T cells (CD3+CD8+)	29.15±9.0 446±226	27.81±6.59 467±215	25.57±7.38 439±237	29.76±5.97 624±167	0.446 0.003	0.146 0.003
CD4/CD8 ratio	1.52±0.74	1.47±0.51	1.72±0.94	1.65±0.48	> 0.05	>0.05
B cells (CD19+)	12.75±4.99 174±85	12.95±5.06 208±102	12.95±4.75 226±6.08	9.24±2.81 192±82	0.004 0.643	0.000 0.576
NK cells [CD3-(CD16+56)+]	14.1±5.33 209±156	14.83±5.63 252±137	16.07±6.08 263±143.0	14.00±6.41 289±149	0.804 0.026	0.49 0.357
NKT cells [CD3+ (CD16+56)+]	8.85±4.75 139±116	10.5±5.74 169±104	9.3±5.21 143±84	6.56±4.68 137±113	0.09 0.991	0.000 0.068
CD57+CD8- cells	10.1±6.06 156±130	14.17±7.2 232±123	11.71±6.94 206±159	5.30±3.77 115±99	0.003 0.255	0.000 0.000
CD57+CD8+ cells	14.6±7.3 233±181	15.5±7.03 255±165	14.76±6.1 254±188	12.74±6.20 267±151	0.412 0.283	0.149 0.4
CD8+CD11+ cells	12.4±6.05 195±148	14.62±6.59 242±148	14.46±6.36 242±136	10.71±4.09 220±104	0.775 0.196	0.052 0.767
CD8+CD11b-cells	19.9±6.59 302±189	16.36±5.06 276±140	15.41±5.78 260±141	19.30±6.10 388±138	0.707 0.042	0.1 0.009
CD8+CD28+ cells	15.6±4.24 231±118	14.66±6.3 249±137	13.45±3.93 228±107	17.34±3.80 340±115	0.5 0.014	0.047 0.026
CD8+CD28- cells	18.4±10.64 298±272	16.1±7.1 296±198	16.06±6.36 270±189	12.58±5.89 245±141	0.145 0.750	0.112 0.483
CD8+CD38+ cells	16.1±8.95 238±178	14.65±6.38 230±115	17.22±7.64 267±149	21.70±7.04 425±123	0.016 0.001	0.001 0.000
CD8+HLA-DR+ cells	7.2±6.1 102±88	5.4±3.07 89±54	5.1±3.26 83±66.0	6.78±4.14 132±70	0.769 0.106	0.241 0.026

* cells/μl

healthy controls. In both groups of treated patients we found the following statistically significant differences as compared to healthy controls: low total T lymphocytes, CD3+CD4+ and CD8+CD38+ subsets, increased relative numbers of B (CD19+) and CD57+CD8- cells and decreased absolute values of suppressor-cytotoxic (CD3+CD8+) and cytotoxic (CD8+CD11b-, CD8+CD28+) T-cell subsets. Few differences were observed between both patients'

groups: lower relative (p=0.028) and absolute (p=0.011) values of CD57+CD8- cells, and higher percentage (p=0.03) of cytotoxic CD8+CD11b- cells in patients treated with IFN compared to those treated with NA. Additionally, comparing the immune parameters of treated patients with those of newly diagnosed (treatment-naïve) with CHB, we found statistically significant differences which consisted of higher relative values of suppressor-

Figure 1. Representative flowcytometric dot plot analysis showing CD8+CD28+ and CD8+CD28- cell subpopulations in patients with chronic hepatitis B, spontaneously recovered and healthy individuals.

Figure 2. Suppressor-cytotoxic T cells (CD3+CD8+) in patients with chronic hepatitis B (group 1- treatment naïve patients; group 2-patients treated with IFN; group 3-patients treated with NA, group 4-healthy controls).

Figure 3. Cytotoxic T-cell subsets (CD8+CD11b-) in patients with chronic HBV infection. **A)** Representative flowcytometric dot plot analysis of cytotoxic T-cell subsets in defined groups: group 1 - treatment naïve patients; group 2 - patients treated with IFN; group 3 - patients treated with NA, group 4 - healthy controls. **B)** Percentage of cytotoxic T-cell subsets in the same groups.

cytotoxic (CD3+CD8+) T lymphocytes (**Fig. 2**) mainly due to higher cytotoxic CD8+CD11b- subset in treated patients (**Fig. 3**). The increase of effector (cytotoxic) cells was more pronounced in the group of interferon treated patients. Analysis of the data showed that as a result of ongoing treatment the percentage of these T-cell subpopulations approached the reference values with their absolute number remaining low.

DISCUSSION

Detection of HBsAg is the first serological marker of hepatitis B infection and its persistence in patients for more than six months is an indication of a chronic infection. However, the majority of patients cleared the virus and were categorized as spontaneously recovered. The antiviral immune response, particularly the T cell arm, is an important factor for the outcome of infection; it has been shown that chronic HBV infection correlates with the occurrence of dysfunctional immune responses.¹¹

Although many studies have studied the role of changes in the immune responses during HBV infection, some questions remain unanswered such as the relationship between the changes in the immune cells subpopulations and disease outcome, the relationships of the latter with HBV replication activity as well as the influence of antiviral therapy. Taking in consideration that the intensity and type of host antiviral immune response are vital for the disease course and outcome we have investigated the changes in immune cells populations and subpopulations in HBV infected patients. With this approach we aimed to help the assessment of the success of viral elimination or response to therapeutic strategies. Summarizing our results we can conclude that in patients with chronic hepatitis B the alterations in immune responses concern both acquired and innate immunity. The observed deficiency of T lymphocytes and their helper-inducer (CD3+CD4+) subset was consistent with the results of other researchers.¹²⁻¹⁴ However, the data on changes in suppressor-cytotoxic (CD3+CD8+) T cells in HBV infection are contradictory. Like other researchers^{14,15}, we have found lower number of suppressor-cytotoxic T-cell subpopulations mainly related with a deficiency of effector (cytotoxic) subsets in treatment naïve patients with CHB. Taking into consideration the importance of cytotoxic T cells response for suppression of HBV replication and viral elimination, Mukherjee et al.¹⁶ assumed that the selective reduction of cytotoxic (CD8+),

but not of helper (CD4+) T cells may be indicative of persistent HBV infection. Our finding also supports the suggested impairment of the effector arm of the adaptive immunity responsible for the effective viral elimination. Like Sun et al.¹⁷ we found elevated relative B-cell numbers in all patients' groups compared to healthy subjects, which could be associated with an active specific humoral immune response against HBV. Significant reduction in the number of NK cells was observed in chronic HBV patients compared with healthy controls.^{18,19} The absence of changes in total NK cells [CD3-(CD16+56)+] in our study could be due to the fact that these cells are involved in the early, nonspecific immune response against the virus. Little information is available in the literature about the role of other functional cell subpopulations studied by us in HBV infection: CD8+CD38+, CD8+CD28+, CD8+CD28-, CD8+CD57+, CD57+CD8-, CD8+CD11b+, CD8+CD11b-, CD8+HLA-DR+, [CD16+(CD3+56)+]. Cao et al.²⁰ found an increased proportion, but not absolute number of activated CD8+CD38+ T cells in patients with chronic hepatitis B in comparison with HBV carriers and healthy controls, and the percentage decreases to normal values as a result of NA therapy. However, the decrease of these cells observed in our CHB patients did not support the above observation. The same authors reported a deficiency in absolute values of CD8+CD28+ cells with effector and/or memory functions which was consistent with our data. It is noteworthy that the data of Li et al.²¹ demonstrating high level of CD8+CD28- subpopulations and low CD28+/CD28- ratio in the CD8+ T cell population in patients with hepatitis B in comparison to healthy controls. Since CD8+CD28+ T cells participate in virus clearance a decrease in CD28 expression may lead to T cell dysfunction, lower CTL activity and difficulty to clear HBV infection.²¹ Discrepancies between our results and those from other investigators can be explained by the fact that the chronic HBV infection has a dynamic course and depending on the disease progression different immune disturbances could be observed.

On the other hand, after spontaneous recovery from HBV infection the immune parameters usually return to normal, indicating a recovery of the patient's immune reactivity.²² It is interesting to note the elevated activated T cells (CD3+HLA-DR+, CD8+CD38+) and NKT cells found in our SR individuals. In connection with this it should

be mentioned that Yu et al.²³ showed a significantly higher proportion of CD8⁺CD38⁺ cells at week 24 of Peg-IFN α -2a treatment in responders as compared to non-responders to therapy. The authors explained this finding with the fact that an increased expression of CD38 on CD8⁺ lymphocytes is suggestive of intensive cellular immunity in CHB patients that may promote the HBsAg clearance.²³ Our data also suggest that antiviral therapy influences the cellular immune response, resulting in the recovery of its effector integrity which is more pronounced in patients treated with interferons. The antiviral therapy can overcome the hypo-responsiveness of CD8⁺ T cells (including effector cytotoxic T-cell subsets) in individuals with CHB suggesting that these T-cell subpopulations exist, but are suppressed in infected patients.²⁴ Consistent to this are the results of Li et al.²¹ for increased proportions of CD8⁺CD28⁺ antigen-primed cytotoxic T cells and CD8⁺CD28⁺/CD8⁺CD28⁻ T cells ratio, and decreased levels of CD8⁺CD28⁻ suppressor T cells in chronic HBV patients given effective therapy with Peg-IFN- α .

In conclusion, our results showed that the surface markers used to define immune cell populations and subpopulations by multicolor flowcytometry are sufficiently informative for evaluation of both the innate and acquired immune response of patients with HBV infection. Therefore, our study of cell populations in patients with different course of HBV infection could be beneficial for the differentiation of immune profiles characteristic for chronic hepatitis B or recovery from infection. Moreover, the immune profiles investigated in treated CHB patients may be used as biomarkers for estimating the effect of antiviral therapy. Future studies including expansion of the set of immunological markers by testing additional cell subpopulations (e.g. Treg) and markers such as CD27, TCR $\gamma\delta$ etc. will allow more complete and detailed evaluation of the cellular immune response in HBV and other viral infections.

ACKNOWLEDGEMENTS

This study was supported by a grant from the National Scientific Fund (DTK02/12-2009).

CONFLICT OF INTERESTS

The authors have no conflict of interest to disclose.

REFERENCES

1. World Health Organization. Hepatitis B. Updated July 2015, World Health Organization Fact Sheet №204. Available from: <http://www.who.int/media-centre/factsheets/fs204/en>.
2. Schweitzer A, Horn J, Mikolajczyk RT, et al. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet* 2015;386(10003):1546-55.
3. Chisari FV, Isogawa M, Wieland SF. Pathogenesis of hepatitis B virus infection. *Pathologie Biologie* 2010;58(4):258-66.
4. Landay A, Gartland GL, Clement LT. Characterization of a phenotypically distinct subpopulation of Leu-2⁺ cells that suppresses T cell proliferative responses. *J Immunol* 1983;131(6):2757-61.
5. Clement LT, Dagg MK, Landay A. Characterization of human lymphocyte subpopulations: alloreactive cytotoxic T-lymphocyte precursor and effector cells are phenotypically distinct from Leu 2⁺ suppressor cells. *J Clin Immunol* 1984;4(5):395-402.
6. Arosa FA. CD8⁺CD28⁻ T cells: certainties and uncertainties of a prevalent human T-cell subset. *Immunol Cell Biol* 2002;80(1):1-13.
7. Strioga M, Pasukoniene V, Characiejus D. CD8⁺CD28⁻ and CD8⁺CD57⁺ T cells and their role in health and disease. *Immunology* 2011;134(1):17-32.
8. Coppo P, Buffet M, Féger F, et al. Polyclonal CD8⁺/CD57⁺ T cell expansions: clinical significance. *Presse Med* 2013;42(3):327-37.
9. Nielsen CM, White MJ, Goodier MR, et al. Functional significance of CD57 expression on human NK cells and relevance to disease. *Front Immunol* 2013;4:422.
10. Mehta K, Shahid U, Malavasi F. Human CD38, a cell-surface protein with multiple functions. *FASEB J* 1996;10(12):1408-17.
11. Bertoletti A, Gehring AJ. The immune response during hepatitis B virus infection. *Journal of General Virology* 2006;87(Pt6):1439-49.
12. You J, Sriplung H, Geater A, et al. Hepatitis B virus DNA is more powerful than HBeAg in predicting peripheral T-lymphocyte subpopulations in chronic HBV-infected individuals with normal liver function tests. *World J Gastroenterol* 2008;14(23):3710-8.
13. You J, Zhuang L, Zhang YF, et al. Peripheral T-lymphocyte subpopulations in different clinical stages of chronic HBV infection correlate with HBV load. *World J Gastroenterol* 2009;15(27):3382-93.
14. Xibing G, Xiaojuan Y, Zhonghua L, et al. Alteration in cellular immunity after chronic hepatitis B deteriorated into severe hepatitis and its significance. *Hepat Mon* 2011;11(10):810-5.
15. Boni C, Penna A, Ogg GS, et al. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. *Hepatology* 2001;33(4):963-71.

16. Mukherjee RM, Reddy PB, Arava J, et al. Relationship between serum HBsAg level, HBV DNA level, and peripheral immune cells in patients with chronic hepatitis B virus infection. *Hepatic Medicine: Evidence and Research* 2010;2:157-62.
17. Sun H, Lv J, Tu Z, et al. Antiviral treatment improves disrupted peripheral B lymphocyte homeostasis in chronic hepatitis B virus-infected patients. *Exp Biol Med* 2013;238(11):1275-83.
18. Zhao PW, Jia FY, Shan YX, et al. Downregulation and altered function of natural killer cells in hepatitis B virus patients treated with entecavir. *Clin Exp Pharmacol Physiol* 2013;40(3):190-6.
19. Li Y, Wang JJ, Gao S, et al. Decreased peripheral natural killer cells activity in the immune activated stage of chronic hepatitis B. *PLoS One* 2014;9(2):e86927. doi: 10.1371/journal.pone.0086927.
20. Cao W, Qiu ZF, Li TS. Parallel decline of CD8+CD38+ lymphocytes and viremia in treated hepatitis B patients. *World J Gastroenterol* 2011;17(17):2191-8.
21. Li X, Kong H, Tian L, et al. Changes of costimulatory molecule CD28 on circulating CD8+ T cells correlate with disease pathogenesis of chronic hepatitis B. *Biomed Res Int* 2014(5):423181. doi: 10.1155/2014/423181.
22. You J, Zhuang L, Zhang YF, et al. Peripheral T-lymphocyte subpopulations in different clinical stages of chronic HBV infection correlate with HBV load. *World J Gastroenterol* 2009;15(27):3382-93.
23. Yu HB, Ma LN, Liu YL, et al. Changes in lymphocyte surface expression of CD8 and CD38 molecules in peripheral blood of inactive HBsAg carriers following pegylated interferon a-2a therapy. *Zhonghua Gan Zang Bing Za Zhi* 2013;21(12):895-8.
24. Boni C, Penna A, Bertoletti A, et al. Transient restoration of anti-viral T cell responses induced by lamivudine therapy in chronic hepatitis B. *J Hepatol* 2003;39(4):595-605.

Оценка подтипов иммунных клеток в качестве предсказывающего инструмента последствия инфекции гепатита В и ответ на лечение

Снежина М. Кандиларова¹, Атанаска И. Георгиева¹, Анастасия П. Михайлова¹, Марта П. Балева¹, Валентина К. Атанасова¹, Диана В. Петрова², Георги Т. Попов³, Елисавета Й. Наумова¹

¹ Кафедра клинической иммунологии и банк стволовых клеток, Университетская больница „Александровска“, Медицинский университет, София, Болгария

² Клиника пропедевтики внутренних болезней, Университетская больница „Александровска“, Медицинский университет, София, Болгария

³ Клиника инфекционных болезней, Военно-медицинская академия, София, Болгария

Адрес для корреспонденции:

Снежина М. Кандиларова,
Кафедра клинической
иммунологии и банк стволовых
клеток, Университетская
больница „Александровска“,
Медицинский университет -
София, ул. Г. Софийски № 1, 1431
София, Болгария
E-mail: sneji_jm@yahoo.com
Тел: +359 2 9230690

Дата получения: 11 сентября
2015

Дата приемки: 31 августа 2016

Дата онлайн публикации:
29 ноября 2016

Дата публикации: 27 марта
2017

Ключевые слова: HBV
инфекция, клеточный

Введение: Иммунный ответ пациентов является одним из важнейших факторов, влияющих на искоренение или хронификацию HBV и считается, что он ответствен за успех лечения.

Цель: Наше исследование ставит перед собой цель выяснить связаны ли защитные механизмы клеток с протеканием инфекции HBV (спонтанное восстановление [CB] или хронификация [XXB]) и с терапевтическим подходом.

Пациенты и методы: 139 пациентов (118 с XXB, 21 с CB) и 29 здоровых индивидов (ЗИ) прошли иммунофенотипирование методом проточной цитометрии. 65 пациентов не проходили лечение, 20 прошли курс лечения интерферонами и 42 - нуклеозидными/нуклеотидными аналогами.

Результаты: Дефицит Т лимфоцитов, хелпер-промотор (CD3+CD4+), цитокин-супрессор (CD8+CD3+) и цитотоксин (CD8+CD11b-, CD8+CD28+) подклассов активированных Т клеток (CD3+HLA-DR+, CD8+CD38+) и увеличение CD57+CD8- клеток, увеличение процента В лимфоцитов и NKT клеток установлено у XXB пациентов по сравнению с ЗИ. У CB пациентов было установлено увеличение CD8+CD11b+, NKT и активированных Т клеток по сравнению с контрольной группой. Повышенные показатели Т клеток и их подклассов у

иммунный ответ,
иммунофенотипизирование

Образец цитирования:

Kandilarova S, Georgieva AI, Mihaylova AP, Baleva MP, Atanasova VK, Petrova DV, Popov GT, Naumova EJ. Immune cell subsets evaluation as a predictive tool for hepatitis B infection outcome and treatment responsiveness.

Folia Medica 2017;59(1):53-62.
doi: 10.1515/folmed-2017-0008

СВ пациентов по сравнению с ХХВ пациентами являются показателем восстановления клеточного иммунитета у индивидов с вылеченной инфекцией HBV. В обеих группах прошедших лечение пациентов установлено понижение Т лимфоцитов, CD3+CD4+ и CD8+CD38+ подклассов по сравнению с ЗИ. Повышенные пропорции подклассов были установлены у пациентов, прошедших лечение по сравнению с ХХВ пациентами, которые не проходили лечение, более сильно выраженные в группе пациентов, прошедших терапию интерферонами.

Заключение: Наши данные показывают, что клеточные иммунные профили могут иметь прогностическое значение при прогнозировании клинического течения инфекции HBV и при уточнении терапевтического ответа.